CUES FOR CAPTURING THE COMPLEXITY OF EQUINE LOCOMOTION

INEKE HILLIE SMIT

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General introduction

Locomotion is a fundamental part of life. The ability to move from one place to another enables animals to interact with their environment, making it an essential factor for natural behaviour, health and welfare. Hence, it is unsurprising that there has been an interest in how animals move since the very dawn of science. The approach towards the study of locomotion has followed the development of science in general. As such, in the days of Antiquity the approach was mainly observational and it was only after the Renaissance, with increasing means and technologies, that experimental studies slowly became mainstream. However, locomotion is incredibly complex and as a result, the scientific approach has been strongly reductionist in character. However, in the past decennia, we have seen an exponential growth of relevant technologies and computational power, which allows for an increase of the comprehensiveness of the traditional reductionist approach. With that, we can slowly try to further understand how locomotion truly works.

This thesis hopes to contribute to the development of an increasingly comprehensive approach to study locomotion. This is done by applying existing techniques to characterise differences between breeds, investigate less common gaits and explore how locomotor-related pain is expressed in different contexts. In addition, considerations to expand the methodological toolbox in the disciplines of both kinematics and muscle function are presented. The subject of this study is the horse, one of the rare species domesticated for its locomotor potential and, in terms of this potential, the most studied non-human animal species. To put this work in a broader context, a brief historical overview of equine locomotion research is given. This is followed by concise synopses of the basics of four-legged locomotion, the current technologies in, and applications of locomotor science in horses, and developments in the digitalisation of movement.

A brief history of equine locomotion research

The first known person to have described locomotion patterns of horses, amongst other species, was the Greek philosopher and natural scientist Aristotle (384 – 322 BCE) in his work "*De motu animalium*". His approach may not have been experimental, still his observations and resulting records of the walking horse were quite accurate. Much later, in the 17th century, the Italian physiologist and mathematician Giovanni Alfonso Borelli wrote his masterpiece with the same title. Borelli's "*De motu animalium*" contains the first descriptions of the musculoskeletal system of animals from a mechanical point of view.[1] With this work it became evident that the science of mechanics needed to be applied to the study of human and animal locomotion in order to understand motor control. This laid to the foundations for understanding how these movements were produced.

In the 19th century, the first 'golden age' of equine locomotion research began. Scientists realised that human senses are insufficient in terms of resolution, reliability and repeatability, to have them serve as scientific instruments for the study of locomotion. With the technological developments of that time, new instruments became available that could describe and capture locomotion. Among the first to apply these instruments to study horses were Eadweard Muybridge, and Etienne-Jules Marey. Muybridge is still famous for his photographs of the cantering horse.[2] Marey may be less well-known today, though he has been equally important for the understanding of animal locomotion with his pneumatic device for the determination of footfall patterns and other inventions such as the 'photographic gun'[3]. The pioneering work of Muybridge and Marey found many followers and progress in the field was great. However, this development was rather abruptly halted by the outbreak of World War II and the following steep decline of the use of horses due to the rapid mechanisation of society. It was the comeback of the horse as a sports and leisure animal in the mid-1960s that, helped by technological developments, led to the 'second golden age' of equine locomotion research from the early 1970s onwards.[4]

Since then, the field of equine biomechanics has blossomed. Over time, the study of equine locomotion has greatly progressed from a descriptive science to one based on quantitative measurements. Significant works have been produced in both the areas of fundamental and applied sciences and more sophisticated equipment and methods to analyse data have become available to capture and digitalise equine locomotion. These developments have paved the way for performing increasingly advanced experiments that yield a much better, and continuously improving, understanding of body function during locomotion.

The basics of quadrupedal locomotion

The locomotor apparatus is a complex system composed of bones, joints, ligaments and muscles, steered by the nervous system. The act of locomotion is defined by a precise linking of multiarticular actions produced by muscle synergies in order to respond to and overcome the forces working on the body. The simplified goal of locomotion can be seen as the displacement of the body centre of mass through space. To do this, the moving limbs generate propulsive forces that subsequently result in displacement of the body.[5] In general, the forelimbs serve a primordial weight-bearing role, whereas the hindlimbs seem to be particularly important to generate the propulsive forces.

A great diversity can exist in equine locomotion patterns because quadrupedal locomotion allows many combinations of inter-limb coordination. In quadrupeds, gaits are typically categorised into 'walking' and 'running' based on their mechanics.[6–8] Two simplified mechanical models are often used to aid the understanding of quadrupedal locomotion (figure 1). The first is the inverted pendulum model, which is used to describe vaulting mechanics related to walking gaits. The second is the spring-mass model, which is used to describe bouncing mechanics related to running gaits. During walking, the centre of mass rotates around the foot, or hoof, over a non-compliant limb.[9–11] In the first half of the stance phase of a limb, the centre of mass rises, converting its kinetic energy into potential energy. The loading response of the limb takes place when the contralateral hoof leaves the ground. In the second half of the stance phase, the potential energy is converted back into kinetic energy as the centre of mass descends. As such, kinetic and potential energy are out of phase. In contrast, during running gaits the centre of mass reaches its lowest point at mid-stance. This is due to the compliance of the limbs, where energy is stored in elastic parts of the limbs, such as tendons.[12–14] In the first half of the stance phase, the centre of mass descends and kinetic and potential energy are converted into elastic energy. At midstance, the peak of the loading response takes place. In the second half of the stance phase, the elastic energy is converted back into kinetic and potential energy as the centre of mass rises again.

Quadrupedal gaits can alternatively be classified into symmetrical and asymmetrical gaits[7,8] based on the sequence of footfalls. The main gaits that are studied in the field of equine locomotion are the walk and the trot, which are both symmetrical gaits, and the canter which is an asymmetrical gait (figure 2). Some horses are able to perform additional symmetrical gaits as a result of genetic mutations related to the DMRT3 gene.[15,16] These are the tölt and pace for Icelandic horses, paso fino and trocha for Colombian horse breeds and other 'ambling gaits' for a selection of different breeds. Which gait the horse may perform at a given moment in time depends on several parameters such as speed and its associated metabolic cost, personal preference and/or human training.

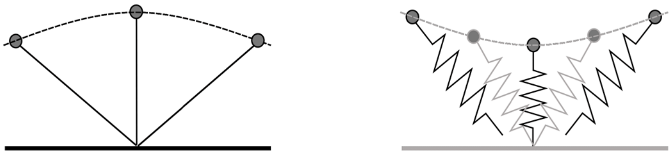


Figure 1. An illustration of the mechanical models that describe A. walking and B. running gaits during a single stance phase. The dashed lines illustrate the vertical path of the centre of mass during stance. Panel A. shows a simple inverted pendulum model, whereas panel B. shows the spring-mass model during one step.

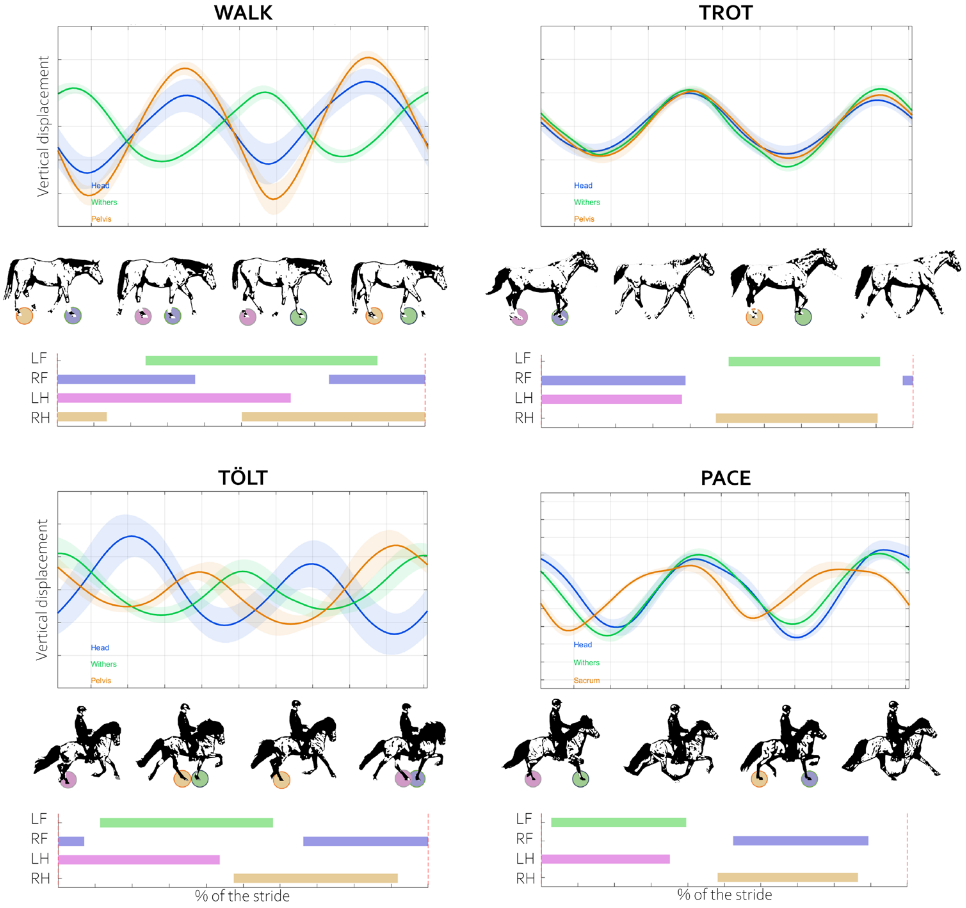
Equine locomotion analysis: why and how?

The current use of the horse as a sports and leisure animal has led to a large economic and societal value. This has incited much research focussed on the understanding of the fundamentals of normal movement and on the identification of changes in locomotor biomechanics due to lameness.[17,18] Lameness is of particular importance as pain is a major cause, which makes this condition an important welfare issue. The methods to study locomotion have evolved over time, closely following technological developments, and can generally be subdivided into methods to study kinetics, kinematics, or muscle function.

Kinetic measurement instruments allow us to describe the forces associated with locomotion. Since the introduction of the force plate in equine locomotion research, kinetic measurements have proven to be very valuable for describing forces related to normal as well as abnormal gaits. Other systems to measure kinetics in horses, such as the instrumented treadmill[19] and force-measuring horseshoes[20], have never been commercialised and their use therefore remains limited. To this day, kinetic measurements are the gold standard for lameness detection by quantifying asymmetrical loading between limbs, though they remain sparsely used. This may be contributed to the cost of the equipment and laborious data collection and processing requirements. However, a recent study involving machine learning methods seems promising for acquiring kinetic data from horses outside of laboratory settings in the future.[21]

Kinematics is a broad term that envelops a branch of biomechanics that focuses on the motion of objects, without reference to the forces which cause the motion. In the 1970s, Fredricson and colleagues[22] introduced high-speed cinematography to capture the 2D kinematics of equine

Figure 2. Illustration of the gaits studied in this thesis. Quarter A. shows the walk, B. the trot, C. the tölt and D. the pace. In each quarter, the upper sub-panel illustrates the vertical head (blue), withers (green) and pelvis (orange) displacement over a stride cycle. In the middle panels, the circles indicate hoof contact with the ground, corresponding to the bottom panels. In the bottom panels, the footfalls of the left front (LF; green), right front (RF; purple), left hind (LH; pink) and right hind (RH; orange) are shown over the mean stride (0%-100%).



locomotion. Later, in the early 1990s, Kübber, Kastner and colleagues started to work with computer-assisted 3D optical motion capture[23,24]. Optical motion capture tracks the position of reflective markers using high-speed infrared cameras and is now considered the gold standard for kinematic analyses. By default, the amount of (stationary) cameras that is used, determines the volume that can measured, and thus, multiple cameras are required to capture consecutive strides. More recently, wearable sensor technology started to gain interest among researchers in this field, as these sensors have the major advantage of being portable. Different sensors (e.g. gyroscopes, accelerometers, global positioning system sensors, etc.) are combined in inertial measurement units (IMUs), which can be attached to relevant anatomical landmarks of the horse in order to capture several aspects of their movement.[25–27] The most recent developments come from the field of computer vision. A single camera (e.g. a smartphone camera) can be used to track specific anatomical landmarks during locomotion, and machine learning algorithms are deployed to help in clinical decision-making. Even though this may be the most user-friendly method, the accuracy is still lower compared to optical motion capture and IMU-based systems and the scope of parameters that can be extracted is still limited.[28]

Muscle function during equine locomotion has been the focus of only a few research groups thus far. Muscle function is often evaluated with needle electromyography (EMG) or surface EMG, which are used to measure different aspects of muscle activation. For locomotion purposes, surface EMG is utilised as this non-invasive technique causes no discomfort when the measured muscle contracts. Surface EMG instruments used to be heavy and wired, prohibiting their use in horses outside of strict laboratory settings (i.e. experiments using a treadmill). Nowadays, multiple wireless systems are available and with that the use of this technology is becoming more common in equine locomotion. In recent years, several experiments have been conducted that looked into changes in muscle activity during specific exercises[29–31] or changes related to lameness[32,33]. However, the use of this technique in horses is still in its infancy and work needs to be done to determine the usability, accuracy and reliability of this technique in non-human animals.

It is particularly the use of kinematic measurement instruments that has taken a flight in the clinical setting over the past decades. The availability of objective measures that quantify locomotion in a clinical setting can be extremely relevant, especially in cases of mild lameness or when performing repeated measurements before and after interventions. In contrast to kinetic and muscle function measurement systems, state-of-the-art kinematic instruments are becoming cheaper and are much more user-friendly than they used to be. Moreover, interpretation of kinematic data is more intuitive, since the data represent the movements we can observe with our own eyes. Current kinematic analysis in a clinical setting heavily depends on within-horse asymmetry, i.e. differences between the left and the right step of a stride. This is logical, since no 'standard' healthy movement pattern exists. For orthopaedic assessments, especially asymmetry parameters relating to the vertical displacement of the head, withers and pelvis have proven to be valuable indicators for lameness.

The digitalisation of locomotion, a dynamic field

In order to capture locomotion, we use measurement instruments that convert analogue signals (e.g. light reflected from an object) into digital signals (e.g. light being sensed by a light sensor in a camera). The analogue signals are continuous in time and amplitude. Digital signals, on the other hand, are time- and amplitude-discrete. How well the digital signal represents the analogue signal it captured, depends on different characteristics of the measurement instrument, primarily the sampling rate and the resolution. Ideally, we need to use a sampling rate greater than twice the bandwidth of the signal we want to capture, according to the Nyquist-Shannon sampling theorem.[34] This means that if a biological event happens at 2Hz, a sampling frequency of at least 4Hz is required to capture it. The resolution of a measurement instrument indicates the number of different, i.e. discrete, values it can produce, which can be expressed in bits. For example, a measurement instrument with a resolution of 8 bits can encode an analogue signal into a digital signal with 256 ($2^8 = 256$) different levels. With optimal sampling rate and resolution of the measurement instruments, the digital signals can be good representations of the analogue signals we want to capture.

From these digital representations, further simplifications are made in order to make the digital signals manageable. The most common simplifications in equine locomotion research are:

- **Normalisation of data,** which facilitates comparisons within and between individual animals. It can be done in multiple ways, but is most often based on time, amplitude, or offset. It manipulates data by reducing (natural) variability, which creates a cohesive version of data entry when analysing data. Nevertheless, normalisation does cause a certain loss in information. With time normalisation, we lose information about the frequency spectrum of a signal; with amplitude normalisation, we lose information about the units the data is collected in; and with offset normalisation, we lose information about the absolute movement in the global coordinate system (e.g., the true location in space).

- **Reduction of the signals to point estimates** that represent a certain aspect of the movement. An example of this is the range of motion, which is the difference between a minimum and maximum value of, for instance, joint angles during a stride. This is a simple, meaningful and discrete value that is easy to interpret and compare between individuals. Other commonly used point estimates are: displacements of body parts and spatio-temporal stride parameters (length, duration, speed, etc.) for kinematics, peak force (vertical, longitudinal and horizontal) for kinetics and amplitude based estimates, such as the average rectified value or root mean square of the signal for muscle activity.

Natural movement is unique and depends on many internal and external factors. The use of simplifications such as normalisation and reduction of signals to point estimates leads to the loss of important information that could otherwise help understand the natural complexity of locomotion. Fortunately, the need for such simplifications is diminishing quickly. Technological developments are rapidly following one another. Reliable, portable and wireless measurement systems are now widely available and increasingly accepted and used for the analysis of equine locomotion. These systems no longer interfere with natural motion of the horse, allowing for more natural data to be collected. Additionally, it is becoming easier to combine or integrate measurement instruments that capture different modalities of locomotion, allowing for the simultaneous capture of kinetics, kinematics and/or muscle function. Also, computational power is drastically increasing, machine learning algorithms are applied to work on datasets too complex for humans to comprehend and more high quality data is fed to models that are becoming progressively better in representing nature. With decreasing size and weight of measurement instruments, increasing signal quality and better processing methods to analyse data, we have by now entered a new phase in which we can work towards an increasingly comprehensive approach of equine locomotion.

Aim and scope of this thesis

The overall aim of this thesis is to contribute to this development. Traditional methodologies are applied to study differences between breeds and gaits in both healthy as well as induced lameness conditions. Then, methodological considerations for analysing kinematic data, as well as to capture electromyographic data are presented.

Chapter 2

This chapter highlights that no horse is the same in terms of their locomotion. Kinematic data of 65 horses of three different breeds were collected using IMUs to describe general differences in locomotion patterns between breeds while performing symmetrical gaits. Emphasis was placed on the Icelandic horse. We assessed timing of peak events and how they related to footfall patterns and mechanics of the different gaits.

Main aim: To compare timing and upper body vertical movement between gaits and breeds.

Chapter 3 and 4

These chapters describe how Icelandic horses adapt to acute fore- and hindlimb lameness during both walk and trot, as well as tölt. The horses were assessed in hand as well as while ridden. Upper body movement and temporal changes in limb kinematics were assessed using IMUs.

Main aim: To evaluate the kinematic changes related to forelimb (**chapter 3**) and hindlimb (**chapter 4**) lameness in walk, trot, and tölt.

Chapter 5

Bilateral and more chronic hindlimb lameness in Shetland ponies is the topic of this study. Optical motion capture was used to capture full body kinematics during trot on a treadmill. In addition, continuous and discrete data analysis were compared to unravel the mechanics of adaptations to bilateral lameness.

Main aim: To compare continuous and discrete data analysis to unravel kinematic adaptations of bilateral hindlimb lameness.

Chapter 6

The study of muscle function in the horse is gaining interest. Therefore, we assessed the effect of electrode location on electromyographic signal quality and activation patterns during trot on the treadmill. Twenty-one muscles were studied in three horses using linear arrays of electrodes.

Main aim: To start the development of recommendations for bipolar electrode location for surface electromyographic measurements in horses.

Chapter 7

Nature is complex and is best studied through collaborative and multidisciplinary networks. Therefore, this chapter is dedicated to open science. Several datasets are described that support the findings of this thesis.

Chapter 8

Finally, in chapter 8, chapters 2 to 7 are summarised and reviewed. In addition, this chapter contains the future perspectives, including the potential implications and applications of the findings of this thesis and their associated benefits and challenges.

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2

Timing of vertical head, withers and pelvis movements relative to the footfalls in different equine gaits and breeds

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Animals. 2022

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Abstract

Knowledge of vertical motion patterns of the axial body segments is a prerequisite for the development of algorithms used in automated detection of lameness. To date, the focus has been on the trot. This study investigates the temporal synchronization between vertical motion of the axial body segments with limb kinematic events in walk and trot across three popular types of sport horses (19 Warmbloods, 23 Iberians, 26 Icelandics) that are known to have different stride kinematics, and it presents novel data describing vertical motion of the axial body segments in tölting and pacing Icelandic horses. Inertial measurement unit sensors recorded limb kinematics, vertical motion of the axial body at all symmetrical gaits that the horse could perform (walk, trot, tölt, pace). Limb kinematics, vertical range of motion and lowest/highest positions of the head, withers and pelvis were calculated. For all gaits except walk and pace, lowest/highest positions of the pelvis and withers were found to be closely related temporally to midstance and start of suspension of the hind/fore quarter, respectively. There were differences in pelvic/withers range of motion between all breeds where the Icelandic horses showed the smallest motion, which may explain why lameness evaluation in this breed is challenging.

Keywords: inertial measurement units; gait; biomechanics; objective motion analysis; lameness

Introduction

An understanding of gait mechanics and normal upper body movement patterns is an essential prerequisite both for visual evaluation and for the development of algorithms to detect lameness based on the presence of movement asymmetries in symmetrical gaits.

Automated lameness detection is based on asymmetrical movement of the axial body segments in symmetrical gaits, which are affected by limb kinematics, ground reaction forces, and whether the gaits use inverted pendulum or spring mass mechanics to conserve energy in the stance phase[1]. These mechanisms will be explained further in the sections on the specific gaits. Previous studies have provided information on breed-specific differences in limb kinematics and kinetics[2], but relatively little is known about the magnitude, range, and timing of movements of the axial body segments.

The present study extends previous work by comparing between-breed reference values for axial body segment kinematics in sound Warmblood, Iberian, and Icelandic horses at walk and trot in hand. Icelandic horses are classified as gaited horses, which implies the ability to perform additional gaits beyond the typical walk, trot and canter/gallop. The amble is a blanket term covering a large number of four-beat gaits performed by gaited horses that have the same footfall sequence as walk but vary in footfall timings and speed of progression. A horse's ability to amble and to pace is largely genetically determined by a nonsense mutation in the DMRT3 gene, also known as the "gait-keeper" gene.[3,4] Icelandic horses that are heterozygous for the DMRT3 mutation are able to tölt, and those that are homozygous can also pace.[3] These gaits are difficult to evaluate using methods applied to walk and trot, and the relatively fast speeds at which they are performed dictate that they are usually evaluated under saddle. Therefore, ridden data for Icelandic horses is presented in order to cover the spectrum of symmetrical gaits in this breed.

Objective evaluation of lameness augments the clinical gait examination by detecting asymmetries that are below the threshold of detection by the human eye[5] while avoiding inherent observer bias[6]. Trot has been the focus of objective lameness evaluation studies due to its symmetrical nature and the higher loading of the limbs compared to walk[7]. A few studies have objectively described the kinematic and kinetic changes in lame horses at walk[7,8], but an attempt to detect lameness objectively during gallop proved unsuccessful[9].

Walk is a four-beat gait with a lateral sequence and successive footfalls separated by approximately 25% of stride duration. As in all symmetrical gaits, the movements of the contralateral limb pairs are out of phase, and there is only a short period of overlap between contact of one limb and lift off of the contralateral limb. The walk uses inverted pendulum dynamics so that the withers and croup are low at hoof-on, vault over the grounded hoof to their highest position during midstance[7], then descend again to lift-off[10,11]. The withers and croup are raised and lowered alternately, with out-of-phase oscillations twice per stride. Vertical head movements are out of phase with the withers and in phase with the croup.[7]

Trot is a two-beat, diagonally coordinated gait with a sinusoidal centre of mass trajectory that is typical of spring-mass mechanics. It incorporates two suspension phases per stride that separate the lift off of one limb from the contact of the contralateral limb so that there is no overlap between them. The trot uses spring-mass mechanics in which the withers and croup are high at the start of stance, descend as the joints flex and limb length shortens in midstance, and rise again at the end of stance[1,11]. The head, withers and croup rise and fall synchronously twice per stride, with minima during the diagonal stance phases and maxima around the time of push-off into the suspension phase.[8] However, the exact timing and variations of these movements have not yet been fully described. Breed differences in limb kinematics of trot have been reported. For example, Iberian horses have a shorter stride length, higher stride frequency and lower dorsoventral displacement and activity than Warmbloods at trot[2], which is likely to affect vertical motion of the axial body segments.

Tölt is a four-beat, lateral sequence, ambling gait without suspension. It has eight limb support phases normally alternating between bipedal and unipedal limb support. Ideally, tölt should have a regular four-beat rhythm with 25% of stride duration elapsing between hoof placements.[12] Due to the frequent periods of single limb support, it has been suggested that it is more difficult for horses to mask lameness in tölt than trot.[13] The mechanical characteristics of tölt are more similar to those of a running gait using spring-mass mechanics but the footfall patterns, and small vertical excursions of the centre of mass are typical of a walking gait.[8] Pattern recognition methods indicated that tölt is more aligned to running than walking gaits.[1] Little is known about relative timing of the head, withers and croup oscillations in tölt.

Pace is a two-beat, laterally synchronised gait with two suspension phases per stride and is classified as a running gait.[12] At high speed, the pace becomes four-beat with hind-first lateral dissociation.[14] Pacing is most often performed by harness-racing horses, and Icelandic horses are somewhat unusual in that they are ridden at pace. Little is known about the timing of the head, withers and croup oscillations in pace.

Differentiation between sound and lame horses has, to date, been based primarily on differences in magnitude of the two minima and maxima of the axial body segments at trot. Differences in head oscillations are used to evaluate forelimb lameness, while differences in croup oscillations are used to evaluate hindlimb lameness.[8, 15-18] In the hind limbs, differences in vertical displacement between the two minima (PDmin) indicate an impact lameness during the first half of stance in which weight acceptance and, therefore, lowering of the body, are reduced on the lame side. Differences between the two maxima (PDmax) indicate a push-off lameness during the second half of stance that propels the pelvis to a lower maximum position after lame limb stance.[19] Vertical withers movements differentiate a primary forelimb lameness from a compensatory head nod shown by horses with a primary hindlimb lameness[20] based on relative timing (phase shift) between head and withers maxima[21]. Information is needed describing the variation in vertical range of motion (ROMz) and timing of the maxima and minima of the head, withers and pelvis in sound horses of different breeds not only at the trot but also at other symmetrical gaits. This information is fundamental to understanding lameness mechanics in different gaits and types of horses.

The first objective is to compare the range and timing of vertical motion of the head, withers, and pelvis in sound Warmblood, Iberian and Icelandic horses performing walk and trot in hand. The second objective is to present key kinematic differences between Warmblood, Iberian and Icelandic horses that may affect visual lameness assessment. The third objective is to present reference data describing the range and timing of vertical movements of the head, withers and croup that are specific to Icelandic horses ridden in walk, trot, tölt and pace.

Across each gait, we hypothesized that (1) absolute timing of limb kinematic variables would vary between breeds; (2) absolute differences in ROMz at all axial locations would be consistently larger in Warmbloods compared to Iberians and larger in Iberians compared to Icelandic horses, not only due to the discrepancy in size but also associated with breed specific gait characteristics; (3) the relative timing of minima and maxima within strides would not vary between breeds.

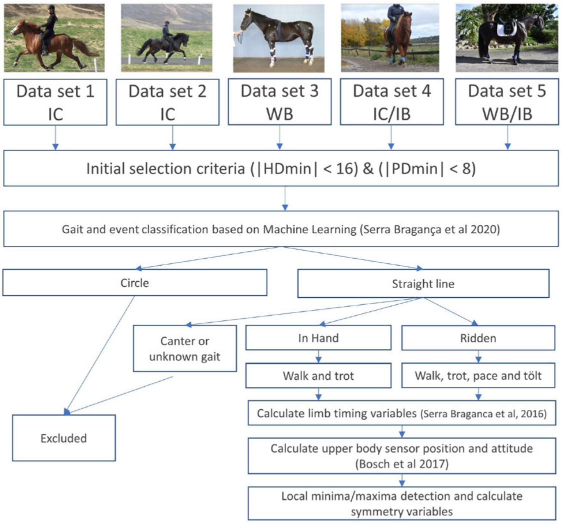
Materials and Methods

Study Design and Study Population

Horses included in this observational study were from previously collected data sets from horses that were sound according to the owner and with only minor objectively measured movement asymmetries that were below predefined thresholds (see the section "Data processing and analysis") when trotting on the straight (Figure 1).

The final data set consisted of horses that were in training at various levels; some were competing and some were leisure horses. Nineteen Warmblood riding horses (11 mares, 8 geldings, age 10 ± 4.2 years, withers height 165.5 ± 7.0 cm) performed 1340 (range per horse 19–202) strides at trot and 1000 (range per horse 19–126) strides at walk in-hand. Twenty-three Iberian horses (10 Lusitano, 13 Pura raza Española, 6 mares, 13 geldings, 4 stallions, age 9 ± 2.5 years, withers height 164.6 ± 4.9 cm) performed 1540 (range per horse 46–119) strides at trot and 1090 (range per horse 19–147) strides at walk in-hand. Twenty-three Icelandic horses (10 mares, 13 geldings, age 10 ± 3.7 years, withers height 141 ± 3.7 cm) performed a total of 815 (range per horse 34–145) strides at trot and 837 (range per horse 27–98) strides at walk in-hand. When ridden 23 horses performed 1920 strides at trot (range per horse 28–203), and 2020 strides at walk (range per horse 25–424), 11 horses performed 410 (range per horse 6–82) strides at pace and 18 horses performed 3100 (range per horse 19–484) strides at tölt.

Figure 1. Schematic representation of the data processing procedure applied for the different data sets. IC: Icelandic horse, WB: Warmblood horse, IB: Iberian horse. HDmin: difference in minimum position of the head between left and right diagonals at trot. PDmin: difference in minimum position of the pelvis between left and right diagonals at trot.



Data Sets

Data were collected in five different research projects between 2017 and 2022 in Sweden, Iceland, the United States of America and the Netherlands, using data from seven IMU sensors (EquiMoves®, The Netherlands)[22] per horse, with a sampling frequency of 500 Hz for measurements in data sets 1–2 and 200 Hz for measurements in data sets 3–5 (Figure 1). The low-g accelerometer in the IMU sensors was set at ±16 g, the high-g accelerometer was set at ±200 g, and the angular velocity was set at 2000 degrees/s. The sensors were attached dorsally to the poll, withers and pelvis in between Tubera sacrale, and to the lateral aspect of each metapodial segment attached on brushing boots. Since the horses were accustomed to wearing brushing boots, they were not disturbed by the limb sensors and none of them reacted to the upper body sensors. Each horse had at least 5 min for acclimatization before measurements started. All horses were measured during walk and trot in hand with different handlers, and the Icelandic horses were also measured at ridden walk, trot, tölt and pace in a straight line on a hard surface (lava sand and packed dirt (Figure 1)).

Data Collection and Selection

Sampled data were transmitted via the gateway to the computer. After each measurement, missing samples on the computer that were not transmitted during live measurement were downloaded from the memory card in the sensors. The software (EquiMoves®) stored the data and pre-computed orientation in log files on the computer. All data retrieved by the software were stored and logged for post-analysis. The data collection was synchronized with video recordings for later data quality checks. The data processing procedure and inclusion criteria are depicted in Figure 1. Algorithms developed previously by machine learning, with an accuracy threshold set to 80%, were used to classify the different gaits[23] and the segments of walk, trot, tölt and pace performed in a straight line were selected for further data analysis. The data were processed using Matlab 2020b (Mathworks, Natick, MA, USA).

Calculated Parameters

For each segment, the variables of interest were calculated, starting by detection of the moments of hoof-on/off for each limb using the data from sensors placed laterally on the metapodial segment of each limb[24] as previously described[22]. Once limb timings had been correctly identified, upper body accelerometer data were double integrated to provide displacement data using previously published methods[25]. Based on the time of left-hind hoof contact, the vertical displacement output of the head, withers and pelvis sensors were segmented into strides and normalized to stride duration, where a stride was defined as the time between consecutive hoof contacts of the left hind limb. Stance normalization was performed based on left front/hind contact and lift-off, respectively. Normalization was set to 100% to enable comparisons between horses with different stride/stance times.

The lowest and highest positions of the head (Hmin/Hmax), withers (Wmin/Wmax) and pelvis (Pmin/Pmax) were calculated from the sensors on the poll, withers and between the tubera sacrale (croup) for each stride, as previously described[22]. Differences between the two minima and between the two maxima for the head (HDmin, HDmax) and pelvis (PDmin, PDmax) were calculated, to determine symmetry during in-hand trot. Horses were excluded if the absolute HDmin and/or HDmax values exceeded 16 mm, and/or if PDmin and PDmax exceeded 8 mm at straight line trot to avoid using horses with marked asymmetry or an underlying lameness.

Diagonal dissociation (time between hoof contacts of the diagonal limbs) was calculated for walk and trot and lateral dissociation (time between hoof contacts of the ipsilateral limbs) was calculated for all gaits and as percentage of stride duration (%StrD). They were defined as positive if hind hoof contact preceded that of the fore hoof. Suspension time was calculated as percentage of stride duration (%StrD) during which none of the hooves were in contact with the ground. It was compared between breeds at trot. Duty factor, which expresses stance phase duration as a proportion of the stride duration, was also calculated.

Statistical Analysis

Results are presented as descriptive statistics. To test the effect of breed (Icelandic vs. Warmblood vs. Iberian) and anatomical location (head, withers and pelvis) on the target variables, linear mixed models were built in R-Studio (version 1.3, Boston, MA, USA). Three models were built: (1) to test the effect of breed and anatomical location on upper body ROMz using horse ID as random effect and the interaction between breed and anatomical location as fixed effect; (2) to test the effect of breed on timing of minima and maxima of head, withers and pelvis positions in relation to stance phase, using breed as fixed effect and horse ID as random effect; and (3) to test the effect of breed on limb kinematic parameters (suspension time, stride duration, diagonal dissociation) using horse ID as random effect and breed as fixed effect. Model results (for ROMz, see Figure 2) are presented as estimated marginal means (EMM) and lower/upper confidence intervals (CI) using the package "emmeans" (version 1.4.5, Boston, MA, USA). Significance was set at $p < 0.05$, and p values were adjusted for multiple comparison using the Tukey method. Model adequacy (normality and constancy of variance) was confirmed using visualisation of the scatter plot residuals vs. fitted values and explanatory variables, respectively, and QQ-plots. The remaining discrete variables are presented as median and interquartile range (IQR).

Results

Temporal Limb Data

Results of temporal limb measurements for the different breeds, gaits and ridden or in-hand conditions are presented in Table 1.

Stride Duration at Walk and Trot

Stride duration was significantly shorter ($p < 0.001$) for Icelandic horses at walk and trot than for Iberian and Warmblood horses. Stride duration was also significantly shorter for Iberian horses than for Warmblood horses at both walk ($p < 0.03$) and trot ($p < 0.013$). No significant differences were seen between in-hand and ridden conditions for the Icelandic horses at walk, but stride duration was significantly longer ($p < 0.001$) during in-hand trot compared with ridden trot. The model outcomes in the form of estimated marginal means are presented in Supplementary Table S1.

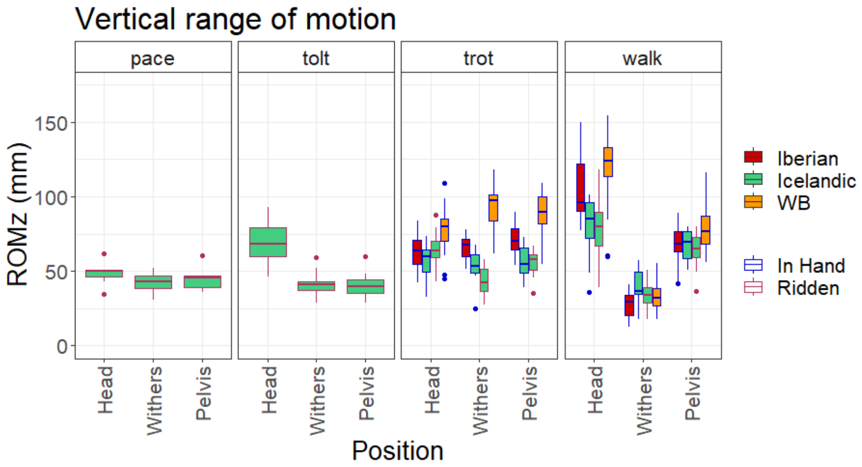


Figure 2. Vertical range of motion (ROMz) of upper body segments for different gaits, breeds and conditions (ridden vs in hand). WB: Warmblood horses.

Suspension Time at Trot

There were no significant differences ($p < 0.991$) in suspension time as a percentage of stride duration between Warmblood horses and Icelandic horses. A trend ($p = 0.06$) towards a longer suspension time was seen for Warmblood horses compared with Iberian horses when trotting in hand. A significantly ($p < 0.020$) longer suspension time was seen for Icelandic horses compared with Iberian horses. For ridden Icelandic horses, suspension time was significantly ($p < 0.001$) shorter compared with in-hand trot. The model outcomes in the form of estimated marginal means are presented in Supplementary Table S1.

Diagonal Dissociation at Trot

There were significant differences ($p < 0.001$, $p < 0.028$) in diagonal dissociation in trot between Iberian and Warmblood horses, and between Iberian and Icelandic horses when trotting in hand. No differences ($p < 0.339$) were seen between Icelandic horses when ridden vs. in-hand trot. The model outcomes in the form of estimated marginal means are presented in Supplementary Table S1.

Vertical Head, Withers and Pelvic Range of Movement

At walk, pairwise breed comparison showed that the Icelandic horses had a smaller ROMz of the head compared to Warmblood and Iberian horses. No significant differences were found for withers and pelvis ROMz between the breeds (Table 2). All within-breed pairwise comparisons of ROMz of the three body segments (head, withers, pelvis) were significant. The head showed the largest ROMz, followed by the pelvis, while the withers showed the smallest.

At trot, Warmblood horses showed significantly larger head ROMz than Iberian and Icelandic horses, but the difference between Icelandic and Iberian horses was not significant. ROMz for withers and pelvis was highest in Warmbloods and lowest in Icelandics with significant differences between all breeds (Table 3). Within-breed comparison of the three body segments showed no differences in Iberian horses. In Icelandic horses, the withers had a lower ROMz than the head or pelvis, while in Warmblood horses, the head had a lower ROMz than the withers or pelvis (Table 3).

Timing of Vertical Movement of Head, Withers and Pelvis

Results of vertical displacement minima/maxima timing of the head, withers and pelvis in relation to the limb events at different gaits are presented in Figures 3–6 and Table 4.

Walk

Descriptive results of the timing of the minima and maxima of the axial body segments (head, withers, pelvis) in relation to the footfall patterns during walk for all horses within the three breeds are presented in Figure 3.

Trot

Descriptive results of the timing of the minima and maxima of the axial body segments (head, withers, pelvis) in relation to the footfall patterns during trot for all horses within the three breeds are presented in Figure 4.

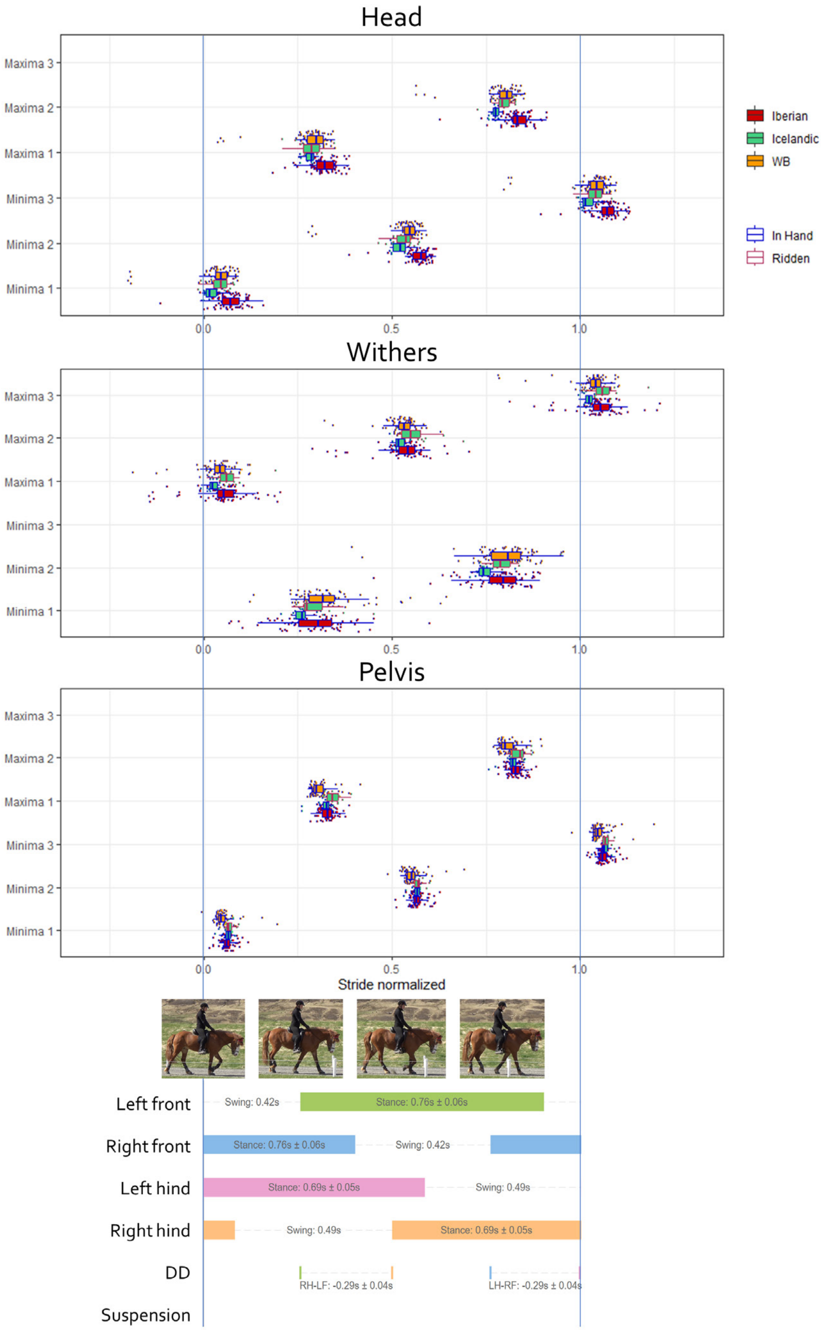


Figure 3. Combined data for the three breeds (Iberian, Icelandic, Warmblood) to show timing of minima (Min) and maxima (Max) on y-axis of the head, withers and pelvis during a normalized walk stride, including data from in hand and ridden conditions. Each dot represents a stride. Stance bars for each limb indicate mean and standard deviation (SD) of all strides across breeds and conditions. Diagonal dissociation (DA) and SD for right hindlimb (RH)-left forelimb (LF) diagonal and left hindlimb (LH)-right forelimb (RF) diagonal are presented.

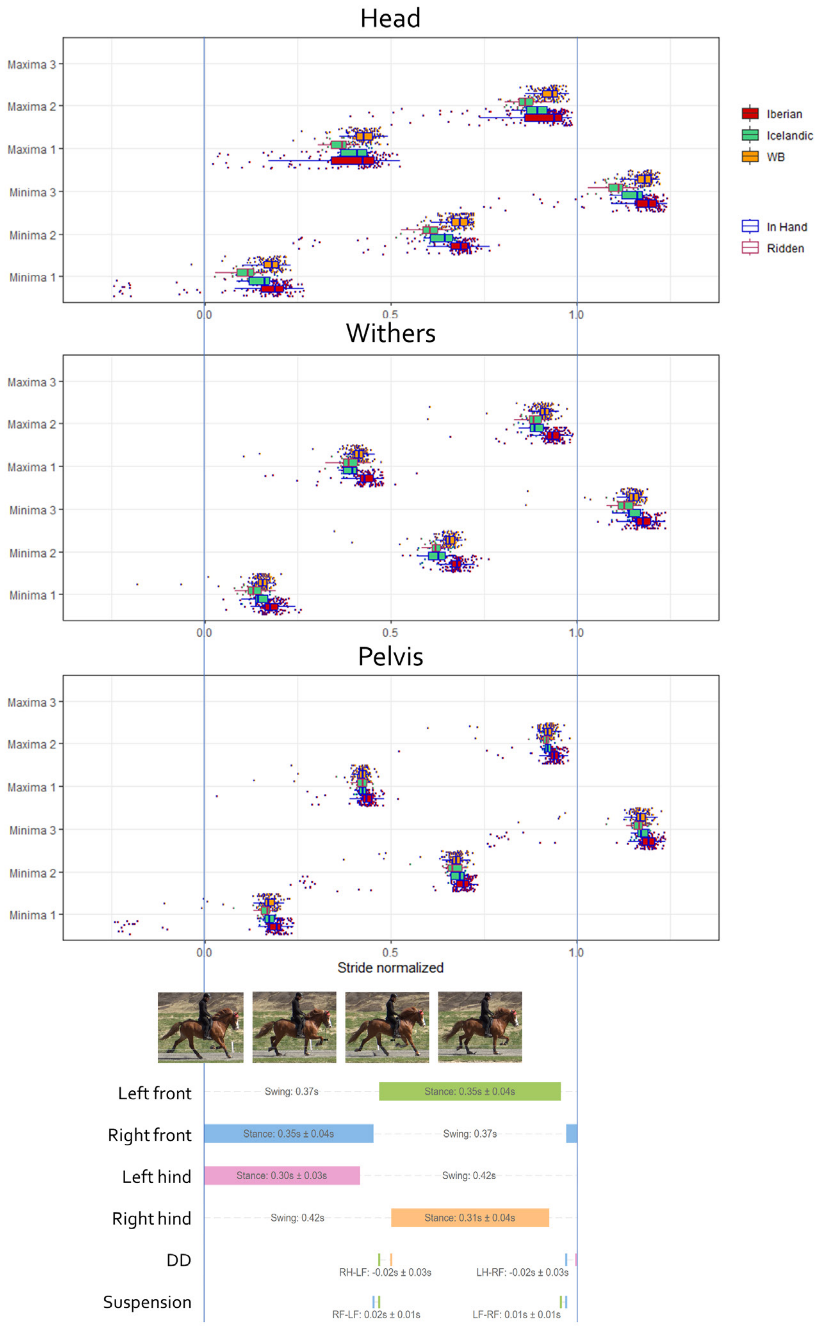


Figure 4. Combined data for the three breeds (Iberian, Icelandic, Warmblood) to show timing of minima (Min) and maxima (Max) on y-axis of the head, withers and pelvis during a normalized trot stride, including data from in hand and ridden conditions. Each dot represents a stride. Stance bars for each limb indicate mean and standard deviation (SD) of all strides across breeds and conditions. Diagonal dissociation (DD) and SD for right hindlimb (RH)-left forelimb (LF) diagonal and left hindlimb (LH)-right forelimb (RF) diagonal are presented. Suspension time is also presented.

	Pace	Tölt	Trot				Walk			
	Icelandic Ridden	**Icelandic Ridden**	**Iberian**	**Icelandic In Hand**	**Icelandic Ridden**	**Warmblood**	**Iberian**	**Icelandic In Hand**	**Icelandic Ridden**	**Warmblood**
Stride duration (s)	0.42 (± 0.029)	0.55 (± 0.068)	0.74 (± 0.037)	0.63 (± 0.083)	0.59 (± 0.085)	0.78 (± 0.048)	1.2 (± 0.15)	1.0 (± 0.20)	0.99 (± 0.13)	1.3 (± 0.11)
Diagonal dissociation*			-1.2 (± 3.5)	-4.8 (± 3.3)	-5.7 (± 5.4)	-2.6 (± 4.0)	-23 (± 3.0)	-26 (± 4.0)	-25 (± 2.7)	-25 (± 2.5)
Lateral dissociation*	9.7 (± 4.3)	18 (± 7.7)	49 (± 3.6)	45 (± 2.9)	44 (± 5.5)	47 (± 4.2)	27 (± 2.9)	23 (± 4.0)	25 (± 2.4)	25 (± 2.1)
Suspension*	13 (± 7.6)	0 (± 0)	2.1 (± 4.2)	3.7 (± 4.8)	0.72 (± 3.2)	4.0 (± 5.4)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
Stance Duration FL (s)	0.15 (± 0.018)	0.25 (± 0.061)	0.37 (± 0.051)	0.30 (± 0.052)	0.27 (± 0.060)	0.38 (± 0.042)	0.77 (± 0.13)	0.63 (± 0.13)	0.63 (± 0.11)	0.85 (± 0.090)
Stance Duration HL (s)	0.17 (± 0.0080)	0.25 (± 0.054)	0.32 (± 0.042)	0.27 (± 0.028)	0.28 (± 0.047)	0.31 (± 0.040)	0.71 (± 0.12)	0.58 (± 0.14)	0.59 (± 0.075)	0.74 (± 0.077)
Swing Duration FL (s)	0.28 (± 0.022)	0.30 (± 0.023)	0.37 (± 0.040)	0.34 (± 0.032)	0.31 (± 0.030)	0.39 (± 0.032)	0.43 (± 0.050)	0.38 (± 0.053)	0.37 (± 0.041)	0.45 (± 0.037)
Swing Duration HL (s)	0.26 (± 0.027)	0.30 (± 0.027)	0.42 (± 0.042)	0.37 (± 0.047)	0.32 (± 0.039)	0.46 (± 0.038)	0.48 (± 0.046)	0.43 (± 0.076)	0.41 (± 0.049)	0.55 (± 0.038)
Duty factor FL	0.36 (± 0.022)	0.46 (± 0.059)	0.49 (± 0.060)	0.46 (± 0.048)	0.46 (± 0.053)	0.49 (± 0.038)	0.64 (± 0.032)	0.63 (± 0.022)	0.62 (± 0.025)	0.65 (± 0.026)
Duty factor HL	0.40 (± 0.024)	0.45 (± 0.049)	0.43 (± 0.055)	0.42 (± 0.032)	0.46 (± 0.049)	0.39 (± 0.039)	0.59 (± 0.033)	0.58 (± 0.022)	0.59 (± 0.026)	0.57 (± 0.022)

Table 1. Stride parameters (median and interquartile range (IQR)) for each breed, gait and condition (ridden/in hand). N: number of horses in each category, WB: Warmblood horses. *as % of stride duration; FL: forelimbs, HL: hind limbs. Diagonal dissociation was not calculated for pace and tölt.

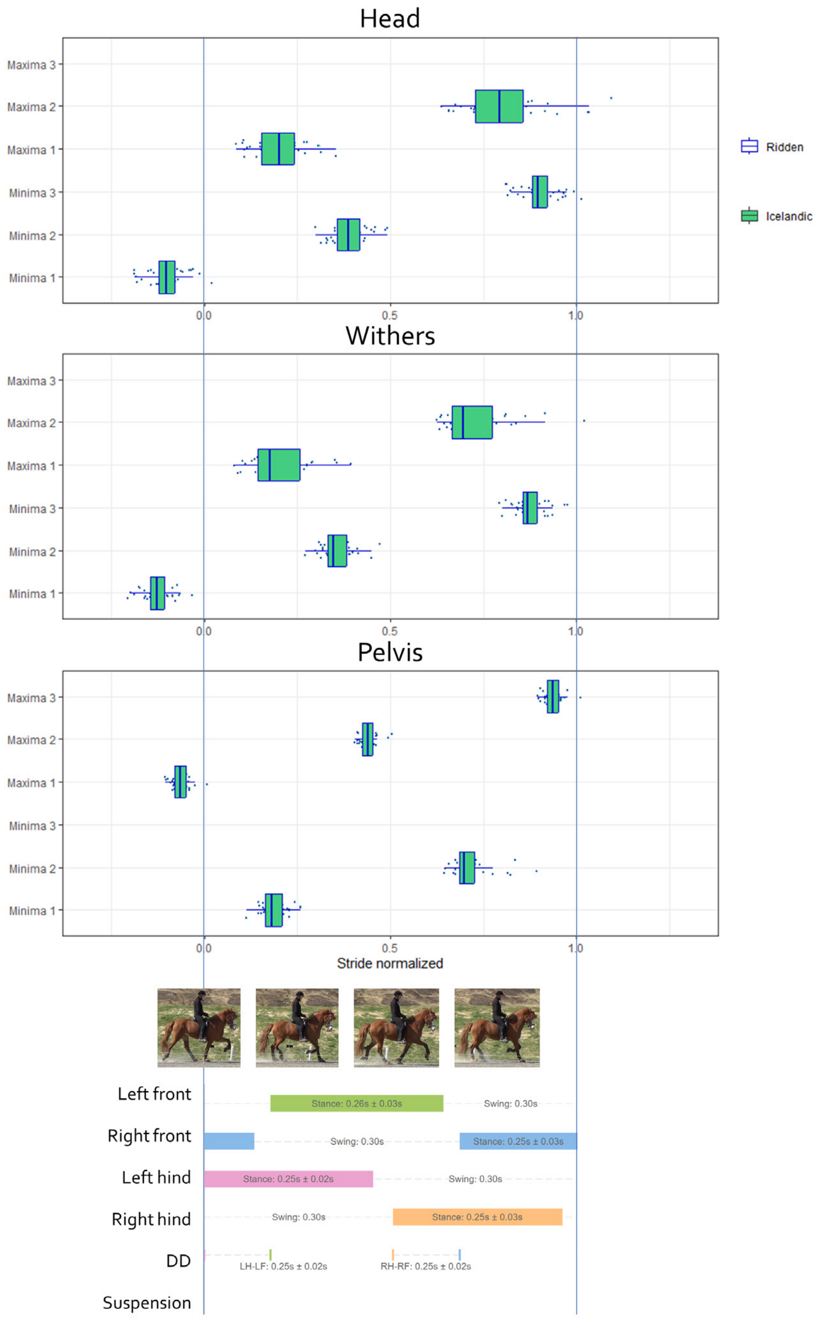


Figure 5. Timing of minima and maxima (y-axis) of the head, withers and pelvis during a normalized tölt stride (0-1.0) for the ridden Icelandic horses. Each dot represents a stride. Stance bars for each limb indicate mean and standard deviation (SD) of all strides. Lateral dissociation (LD) and SD for left hindlimb (LH)-left forelimb (LF) and right hindlimb (RH)-right forelimb (RF) are presented.

Tölt

Descriptive results of the timing of the minima and maxima of the axial body segments (head, withers, pelvis) in relation to the footfall patterns during tölt for the Icelandic horses are presented in Figure 5.

Pace

Descriptive results of the timing of the minima and maxima of the axial body segments (head, withers, pelvis) in relation to the footfall patterns during pace for the Icelandic horses are presented in Figure 6.

Differences in relative timing of minima and maxima between the different body segments (head–withers, head–pelvis and withers–pelvis) in the three breeds when performing different gaits are presented in Table 5 and Figures 7 and 8.

	HEAD			**WITHERS**			**PELVIS**			**Within-breed significant pairwise comparison ($p<0.01$)**
	EMM	**Lower CI**	**Upper CI**	**EMM**	**Lower CI**	**Upper CI**	**EMM**	**Lower CI**	**Upper CI**	
Iberian (n=1090)	104.7	95.3	114	26.8	17.4	36.2	68	58.6	77.3	All
Icelandic (n=837)	79.3	71.3	87.4	36	28	44	65.8	57.8	73.8	All
WB (n=1000)	117.1	107	127.2	33.4	23.3	43.5	78.8	68.7	88.9	All
Between-breed significant pairwise comparison ($p<0.001$)		All			None			None		

Table 2. Vertical range of motion (mm) at walk in hand for head, withers and pelvis. Results presented are estimated marginal means (EMM) with confidence intervals (CI) from the linear mixed model with pairwise comparisons. n=number of strides.

	HEAD			**WITHERS**			**PELVIS**			**Within-breed significant pairwise comparison ($p<0.01$)**
	EMM	**Lower CI**	**Upper CI**	**EMM**	**Lower CI**	**Upper CI**	**EMM**	**Lower CI**	**Upper CI**	
Iberian (n=1540)	63.7	56.8	70.6	66.2	59.3	73.1	70.8	63.9	77.7	None
Icelandic (n=815)	60.7	54.7	66.7	46.8	40.8	52.9	56	50	62	H-W, W-P
WB (n=1340)	78.1	70.5	85.7	92.2	84.6	99.8	89.1	81.5	96.7	H-W,H-P
Between-breed significant pairwise comparison ($p<0.001$)		Icelandic-Warmblood Iberian-Warmblood			All			All		

Table 3. Vertical range of motion (mm) at trot in hand for head (H), withers (W) and pelvis (P). Values presented are estimated marginal means (EMM) with confidence intervals (CI) from the linear mixed model with pairwise comparisons. n=number of strides.

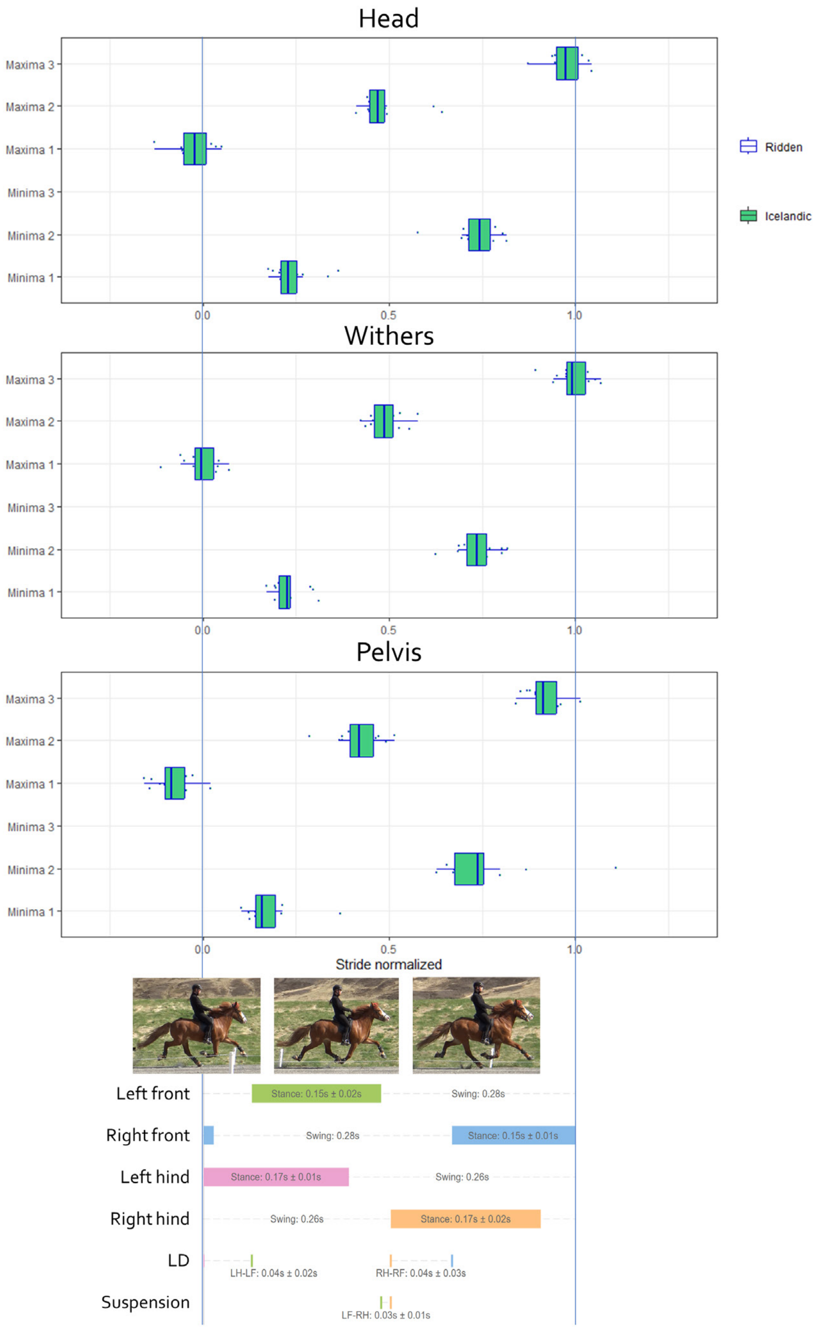


Figure 6. Timing of minima and maxima (y-axis) of the head, withers and pelvis during a normalized pace stride (0-1.0) for the ridden Icelandic horses. Stance bars for each limb indicate mean and standard deviation (SD) of all strides. Lateral dissociation (LD) and SD for left hindlimb (LH)-left forelimb (LF) and right hindlimb (RH)-right forelimb (RF) are presented. Suspension time is also presented.

		Minima **Median (± Interquartile range)**				**Maxima** **Median (± Interquartile range)**			
		Iberian In hand	**Warmblood In hand**	**Icelandic In hand**	**Icelandic Ridden**	**Iberian In hand**	**Warmblood In hand**	**Icelandic In hand**	**Icelandic Ridden**
Head	Walk	48 (±5.4)	47 (±5.5)	46 (±4.6)	46 (±5.5)	88 (±5.6)	87 (±5.5)	86 (±3.6)	88 (±4.7)
	Trot	41 (±9.4)	46 (±7.9)	39 (±5.2)	40 (±13)	92 (±20) D**/E**	97 (±6.6)	92 (±9.1) D**	94 (±11) E**
	Tölt				49 (±15)				4.8 (±20)
	Pace				27 (±12)				95 (±11)
Withers	Walk	5.3 (±14) A**/ B*	13 (±11) A**	4.6 (±5.3) B*	3.7 (±4.7)	42 (±6.6) D**	44 (±4.8)	48 (±5.3) D**	45 (±6.3)
	Trot	39 (±5.0)	40 (±4.4)	42 (±5.6)	41 (±9.8)	91 (±6.7) D**	92 (±4.6) E*	94 (±5.6)	98 (±7.5) D**/E*
	Tölt				39 (±13)				-0.31 (±24)
	Pace				27 (±9.6)				99 (±14)
Pelvis	Walk	10 (±2.5) A**	7.7 (±2.6) A**/B**/C**	11 (±2.4) B***	11(±2.5) C***	55 (±44) D**	51 (±3.3) D**/E**/F**	57 (±3.8) E**	56 (±6.1) F**
	Trot	45 (±7.1)	42 (±6.0)	41 (±6/7)	37(±5.5)	100 (±5.5) D***/E**	110 (±3.8) E**/F**/G***	100 (±6.2) F**	92 (±5.2) D***/G***
	Tölt				40 (±8.7)				97 (±7.1)
	Pace				41 (±13)				100 (±17)

Table 4. Timing of minima and maxima. Values for head and withers expressed as percentage of left forelimb stance and values for pelvis expressed as a percentage of left hindlimb stance. Statistically significant differences between breeds are indicated separately for minima and maxima by pairs of values with the same letters (A-G), with level of significance indicated by asterisks (*p<0.05, **p<0.01, *** p<0.001).

		Difference in Minima as % of stride **Median(± Interquartile Range)**			**Difference in Maxima as % of stride** **Median (±Interquartile Range)**		
		Iberian	**Warmblood**	**Icelandic**	**Iberian**	**Warmblood**	**Icelandic**
Head-Withers	Walk	23 (±6.7) A***	28 (±6.3) A***	23 (±4.4)	-27 (±3.4) A**	-26 (±2.6)	-25 (±3.8) A**
	Trot	-1.6 (±5.4) A***	-3.3 (±2.2) A***/ B**	1.1 (±3.3) B**	-0.53 (±7.7) A***	-2.3 (±2.2) A***	1 (±2.7)
	Tölt			-2.9 (±3.9)			-3.6 (±10)
	Pace			-1.3 (±1.9)			0.9 (±2.2)
Head-Pelvis	Walk	-0.5 (±2.6) A**	-0.7 (±2.9)	3.3 (±3.3) A**	-0.2 (±2.2) A**	-0.4 (±3.1) B**	4.3 (±3.1) A**/B**
	Trot	-0.3 (±3.0)	-1.4 (±2)	3.8 (±4.3)	-0.3 (±8.0)	-1.4 (±2.5) B**	3.7 (±4.7) B**
	Tölt			29 (±4.7)			- 32 (±11)
	Pace			-3.4 (±3.8)			-7 (±7.0)
Withers-Pelvis	Walk	-23 (±7.8) A*	-28 (±7.6) A*/B***	-19 (±3.7) B***	27 (±3.2)	26 (±3,1)	30 (±3.7)
	Trot	1.6 (±2.7) A**	1.8 (±1)	3 (±3.7) A**	0.2 (±1.8)	0.7 (±1.4)	2 (±3.4)
	Tölt			33 (±5.9)			-26 (±12)
	Pace			-2.2 (±9.6)			-8.5 (±6.8)

Table 5. Difference in timing of vertical minima and maxima between different axial body segments (head-withers, head-pelvis, withers-pelvis) in Iberian, Warmblood, and Icelandic horses performing different gaits. Significant differences in pair-wise comparisons between breeds are indicated by the same letters (A, B) and asterisks indicating the level of significance (*p<0.05, **p<0.01, ***p<0.001).

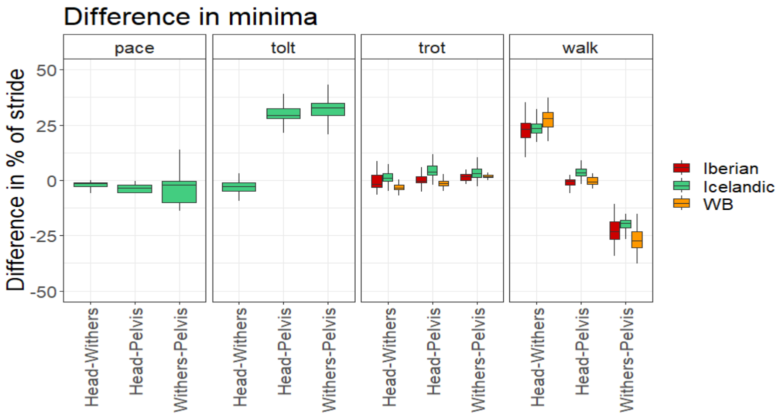


Figure 7. Timing difference in vertical displacement minima between head, withers and pelvis for the different breeds and gaits studied, based on LH hoof-on stride split. WB: Warmblood horses. A positive Head-Withers value indicate that the head reaches the minima first. A positive Head-Pelvis means the head reaches the minima first. A positive Withers-Pelvis means the withers reaches the minima first.

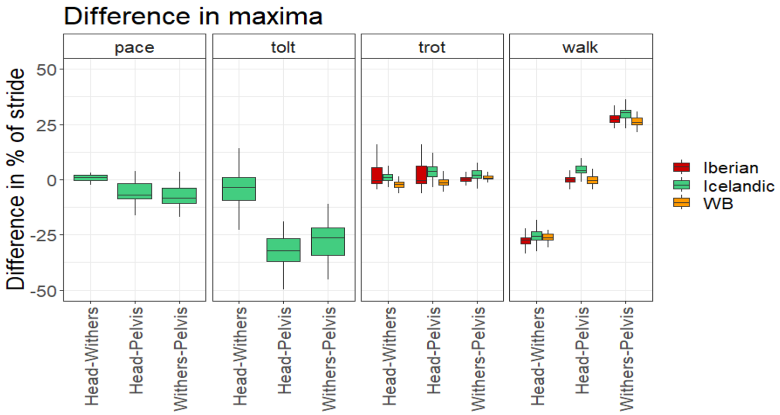


Figure 8. Timing difference in vertical displacement maxima between head, withers and pelvis for the different breeds and gaits studied. WB: Warmblood horses. A positive Head-Withers value indicate that the head reaches the minima first. A positive Head-Pelvis means the head reaches the minima first. A positive Withers-Pelvis means the withers reaches the minima first.

Discussion

This study revealed clear differences not only in limb kinematics but also in upper body kinematics between Icelandic, Warmblood and Iberian horses. It also demonstrated that such differences can be quantified in field conditions using mobile sensor technology. These findings open the way for more kinematic studies involving between- and within- breed comparisons and represent a first step in determination of reference kinematic values. Modern objective lameness assessment methods make use of symmetry values to overcome the lack of absolute reference values[26]; hence, establishing reference values for specific breeds offers the possibility to further standardize gait analysis techniques in the near future, in order to establish breed-specific gait patterns.

At trot, Warmblood horses showed a greater vertical range of motion of the upper body than Icelandic and Iberian horses, which might be expected due to producing relatively larger vertical impulses compared to other breeds[27]. Barrey et al. also found a greater vertical displacement of sternum in Warmblood horses compared to Spanish horses. [2] Our results clearly showed a close temporal relationship between vertical movements of the upper body and the limb stance phases, which is very consistent across breeds at a particular gait. Therefore, it is likely that upper body movement asymmetry, which is used for lameness quantification at trot, can also be a useful variable for lameness evaluation in symmetrical gaits other than trot[26].

Temporal Variables

Stride Duration

Stride duration differed between all breeds at both walk and trot, with Icelandic horses showing the shortest stride duration in hand, although it increased when they were ridden. Warmblood showed the longest stride duration. There were no differences in relative suspension time between Warmblood and the other two breeds when trotted in hand, but Icelandic horses showed a longer suspension time compared to Iberian horses. The short stride duration in Icelandic horses can be related to their shorter limb length, lower body mass, and the consequently higher stride frequency. These characteristics may contribute to making visual lameness assessment in this breed more challenging[28].

Diagonal Dissociation at Trot

On average, horses in the present study showed forelimb-first diagonal dissociation at trot, which agrees with earlier studies on Icelandic horses[29] and Warmblood horses[30] trotting in hand on a treadmill. In Iberian horses, the fore-first diagonal dissociation at trot was shorter than in Warmblood horses and Icelandic horses. Earlier landing of the hind hoof compared with the diagonal forelimb has been observed in previous studies on Warmblood horses and has been associated with higher trot quality.[31] It has also been reported in ridden Warmblood horses performing collected, working, medium and extended trot that diagonal dissociation was shortest during collection.[32] The fore-first dissociation in Icelandic horses may be associated with their lower quality of trot compared with the other breeds and may also be affected by the DMRT3 gene mutation seen in Icelandic horses.[3,4]

Relative Time Shifts between Segments

The vertical minima (Hmin/Wmin/Pmin) occurred around midstance for all gaits except for pace (Hmin/Wmin around 27% of stance) and for Pmin and Wmin at walk, where the lowest positions were reached during dual hindlimb and dual forelimb support, respectively, when the contralateral limbs were maximally spread apart. All Hmax/Wmax/Pmax occurred within the period from the last 15% of stance to the first 10% of the next limb stance phase, except for Pmax and Wmax at walk (42–56% of stance phase). Therefore, changes in vertical movement symmetry for Hmin/Wmin/Pmin are probably good indicators of weight-bearing lameness in tölt and pace. PDmax is likely to be a good indicator of push-off lameness[19] except in walk where the pelvis reaches its highest position during mid-stance. In horses with weight-bearing forelimb lameness during walk, adaptive movement strategies appearing as differences in the lowest position of the head (HDmin) and withers (WDmin) have been reported.[7] Further studies are needed in hind limb lame horses during walk and in lame horses in gaits other than trot to identify their compensatory lameness strategies, and thereby improve subjective and objective lameness detection.

Timings of vertical minima and maxima of head, withers and pelvis were synchronised for trot and pace. For walk, the maxima and minima of the head and sacrum were in phase, while the withers were around 25% out of phase compared with both head and sacrum. For tölt, timings of the minima and maxima of head and withers were synchronised, while pelvis minima and maxima were around 25% out of phase with head and withers.

Vertical Head, Withers and Pelvis Range of Movement

Different approaches are currently used for lameness quantification. For example, the differences between minima and maxima are used for head (HDmin/HDmax), withers (WDmin/WDmax) and pelvis (PDmin/PDmax) (EquiGait®, EquiMoves®, Q-horse®) or these values are corrected for the amplitude of the second harmonic (Equinosis®)[33]. The lower range of motion of the head observed in this study for Icelandic and Iberian horses compared with Warmblood horses may affect the magnitude of these asymmetry variables differently, but we only know how the range of motion is affected by lameness in Warmblood horses[7]. The range of motion of head, withers and pelvis is also negatively correlated to speed[34], as speed increases the range of motion of head, withers and pelvis decreases. Speed has less influence on movement symmetry variables during straight line trot[28], but if these variables are corrected for vertical range of motion, they will probably also be affected by speed[28]. This is also very relevant when performing visual lameness evaluation, which is based largely on assessment of upper body movement symmetry. If the ROMz is smaller, as observed here for Icelandic horses at trot and for Iberian horses, the capacity of the human eye to detect this motion might be hampered[5], making it more challenging to detect lameness at trot in the Icelandic or Iberian horse compared with the Warmblood horse.

Implications for Lameness Assessment of Different Breeds

A short stride duration, i.e., a high stride frequency, may be one of the factors making visual lameness assessment more challenging.[28] This, in combination with a smaller ROMz at trot, might call into question the accuracy of visual lameness assessment for Icelandic horses, supporting the use of gait analysis methods in this breed. In the present study, Icelandic horses showed a smaller ROMz of the head at walk and of the head, withers and pelvis at trot, which in combination with the higher stride frequency, may also partly explain why lameness evaluation in this breed is regarded as very challenging. In addition, there may be differences in lameness adaptation strategies between breeds due to the DMRT3 gene mutation, which may allow Icelandic horses to alter their footfall pattern more easily than Warmblood horses. Therefore, further studies are needed on lameness compensation in gaited horses.

Inclusion Criteria

For all data sets, the initial inclusion criterion was "sound horse in training", but despite this, many horses presented with a high degree of motion asymmetry as seen in previous studies[35]. Therefore, an additional selection was made for the present study to avoid the influence of obvious lameness on the kinematic variables of interest. The thresholds chosen were slightly above the reference literature on inter-run variability in sound warmblood horses[36,37] but within Thoroughbred repeatability values[21], taking into account the substantial systematic differences between measuring systems[38]. Data were also selected by using previously developed gait classification algorithms[23] for the different gaits. The neural network algorithm combines several of the parameters tested to make a decision regarding whether a particular stride is classified as a specific gait (walk, trot, tölt or pace). For this study, we set a classification threshold of 80%, meaning that any strides used by the combination of the selected variables to predict the gait[23] which yielded a classification with accuracy <80% were removed. This could have affected our results by a selection bias towards only including strides where horses displayed features with greater weight in the classification, with those horses being overrepresented in the data set. Classification of tölt and pace based on visual labelling of strides from video[23] can also have led to biased selection of specific features that were only selected initially from video observation. Based on the 80% accuracy thresholds, no gaits from the horses were excluded from the final results due to gait classification, meaning that all 26 Icelandic, 19 Warmblood and 23 Iberian horses are represented in the results.

Limitations

This study includes a limited number of horses per breed. Consequently, factors that could have been interesting to study in relation to the presented data, such as conformation, training level and size, could not be included in our analysis. Due to the multi-centre nature of this study, differences in surface between and within breeds can be expected and might have affected some of the stride temporal variables measured at the different sites[39]. In addition, there is some potential clustering effect within breeds, given that some horses are from the same locations. At the same time, within each breed, data were collected in different countries. Not all data sets contained speed that could be used in the statistical models, and therefore, speed was not taken into account during statistical analysis[30]. The proximal limb angles may influence the stride variables[40] but were not measured in the present study.

Conclusions

Vertical minimum and maximum position of head, withers and pelvis generally occurred around midstance of the forelimb and hindlimb for all gaits except for head and withers in pace and pelvis and withers at walk. The highest vertical position of head, withers and pelvis occurred from the last 15% of stance through the first 10% of the following limb stance phase, except for the withers and pelvis at walk. Icelandic horses showed a smaller vertical range of motion of the head at walk and of the head, withers and pelvis at trot. This, in combination with higher stride frequency, may partly explain why lameness evaluation in this breed is regarded as very challenging.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ani12213053/s1, Table S1: Model results for stride duration at walk and trot, diagonal dissociation and suspension at trot are presented as estimated marginal means (EMM) and lower/upper confidence intervals (CI). Significant pairwise comparisons between breeds (WB = Warmbloods) are presented at the bottom of the tables.

Author Contributions: Conceptualization, T.P., M.R., E.H. and F.S.B.; Methodology, T.P., M.R., E.H., H.M.C., S.J.H. and F.S.B.; Software; I.H.S. and F.S.B.; Formal Analysis, I.H.S. and F.S.B.; Investigation; all authors; Resources, M.R., V.G., S.B., F.S.B. and H.M.C.; Data Curation, I.H.S., F.S.B. and V.G.; Writing—Original Draft Preparation, M.R., E.H. and F.S.B.; Writing—Review and Editing, M.R., I.H.S., E.P.-S., T.P., V.G., S.B., E.Z., H.M.C., S.J.H., F.S.B. and E.H.; Visualization, F.S.B. and M.R.; Project Administration, M.R., V.G., S.B. and E.H. Funding Acquisition, M.R., E.H., T.P., F.S.B. and S.B. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed written consent was obtained from the owner of each animal. No data on human participants were used in this study.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Adaptation strategies of the Icelandic horse with induced forelimb lameness at walk, trot and tölt

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Abstract

Background and objective: Lameness assessment in the gaited Icelandic horse is complex. We aimed to describe their kinematic and temporal adaptation strategies in response to forelimb lameness at walk, trot and tölt.

Study design: In vivo experiment.

Methods: Ten clinically non-lame Icelandic horses were measured before and after reversible forelimb lameness induction. Upper body and limb kinematics were measured using 11 inertial measurement units mounted on the poll, withers, pelvis (tubera sacrale) and all four limbs and hoofs (Equimoves®, 500 Hz). Horses were measured on a straight line at walk and trot in-hand and at walk, trot and tölt while ridden. Linear mixed models were used to compare baseline and lame conditions (random factor = 'horse'), and results are presented as the difference in estimated marginal means or percentage of change.

Results: Lameness induction significantly ($p < 0.05$) increased head vertical movement asymmetry at walk ($HDmin/HDmax_{HAND}$: 18.8/5.7 mm, $HDmin/HDmax_{RIDDEN}$: 9.8/0.3 mm) and trot ($HDmin/HDmax_{HAND}$: 18.1/7.8 mm, $HDmin/HDmax_{RIDDEN}$: 24.0/9.3 mm). At the tölt, however, HDmin did not change significantly (1.1 mm), but HDmax increased by 11.2 mm ($p < 0.05$). Furthermore, pelvis vertical movement asymmetry (PDmax) increased by 4.9 mm, sound side dissociation decreased (-8.3%), and sound diagonal dissociation increased (6.5%). Other temporal stride variables were also affected, such as increased stance duration of both forelimbs at walk, tölt and in-hand trot.

Main limitations: Only one degree of lameness (mild) was induced with an acute lameness model.

Conclusions: Classical forelimb lameness metrics, such as vertical head and withers movement asymmetry, were less valuable at tölt compared to walk and trot, except for HDmax. Therefore, it is advised to primarily use the walk and trot to detect and quantify forelimb lameness in the Icelandic horse.

Keywords: asymmetry; equine biomechanics; gaited horse; inertial measurement units; kinematics

Introduction

With its ability to tölt, the Icelandic horse has been rising in popularity among recreational and competitive riders. Due to their growing presence, there is also an increased need for knowledge regarding lameness adaptations in this breed. Clinicians often testify that assessing lameness in Icelandic horses is particularly challenging. This may be related to their high stride frequencies,[1–3] lower vertical range of motion of head, withers and pelvis compared to warmbloods at trot[3] and/or the diversity of footfall patterns the Icelandic horse can show[1] as a consequence of the high prevalence of the known mutation of the *DMRT3* gene.[4] Especially during tölt, a four-beat running gait either shown as a pure (while ridden) or as a mixed gait (sometimes encountered during lameness exams), our understanding of how Icelandic horses adapt to lameness is minimal.

Upper body vertical movement asymmetry parameters at the trot are currently the primary resource for quantitative lameness assessment in clinical practice.[5] The most commonly used asymmetry parameters to quantify lameness are the differences between the two local vertical displacement minima/maxima within each stride for the head (HD_{min}/ HD_{max}), withers (WD_{min}/WD_{max}) and pelvis (PD_{min}/PD_{max}). These movement asymmetries in lame horses are related to weight redistribution between limbs,[6–9] where the peak vertical ground reaction force (i.e., limb loading; GRFz) of the lame limb is reduced. Head and withers asymmetry parameters have also been shown to be reliable indicators of forelimb lameness at walk.[10] Therefore, these asymmetry parameters seem logical candidates for lameness evaluation at tölt. However, at the tölt, the footfall pattern, and thereby weight distribution between limbs, differs from walk and trot.[1,11,12] While trot is a symmetrical two-beat diagonal gait,[13] walk and tölt are symmetrical four-beat gaits with lateral sequences of footfalls.[1,13] In contrast, tölt mechanics are closer to those of running gaits such as trot,[12,14] whereas the walk is a clear example of a walking gait.[14] Furthermore, true tölt (TT) has no suspension phase, and uni- and bipedal support phases should alternate with a limb phase of approximately 25%.[1,13] Therefore, lameness metrics studied for walk and trot might not directly apply to tölt.

Controversy exists among studies regarding the gait dynamics of Icelandic horses at tölt. The sequential placement of the limbs at tölt reduces the peak vertical accelerations of the body centre of mass,[15,16] such that the transition from two-beat trot to four-beat tölt may be a possible lameness adaptation strategy without a speed reduction. Conversely, it has been suggested that to decrease the loading of the lame limb at tölt may be less effective compared to the walk and trot as there are periods of unipedal support for each limb.[12] When peak GRFz are compared between trot and tölt, one study reports lower forelimb peak GRFz at tölt compared to overground trot,[12] while another study reported higher peak GRFz for the forelimbs at tölt compared to trot on a treadmill.[17]

This study aimed to describe the movement pattern changes in response to induced forelimb lameness at walk, trot and tölt during both in-hand and ridden conditions. We hypothesised that Icelandic horses would show vertical movement asymmetry of the head and withers in response to induced forelimb lameness at all gaits. However, these changes were expected to be larger at the faster gaits and smaller with the presence of a rider compared to in-hand conditions.

Materials and methods

Horses

A convenience sample of 10 clinically sound Icelandic horses (3 mares and 7 geldings; age: 7.5 [5–25] years; weight: 384 [370–405] kg; median [range]) in full training were included in this study. The horses were selected from a group of 30 horses, the majority University owned and two of which were privately owned. Horses were included when judged as non-lame based on pre-trial visual and objective lameness measurements (Equinosis) at trot in-hand, as well as clinical examinations of the locomotor apparatus by three experienced orthopaedic veterinarians (E.H., M.R. and F.S.B.). All horses could tölt while ridden (eight were homozygous for the AA genotype of the DMRT3 gene, and two were heterozygote AC).

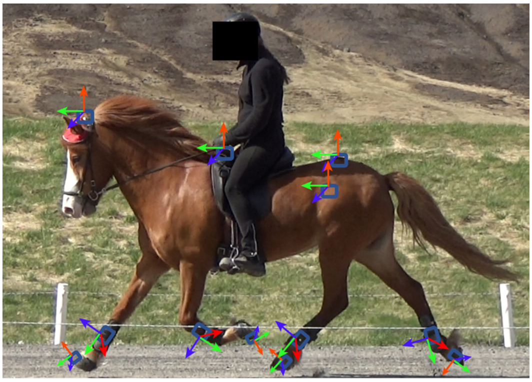
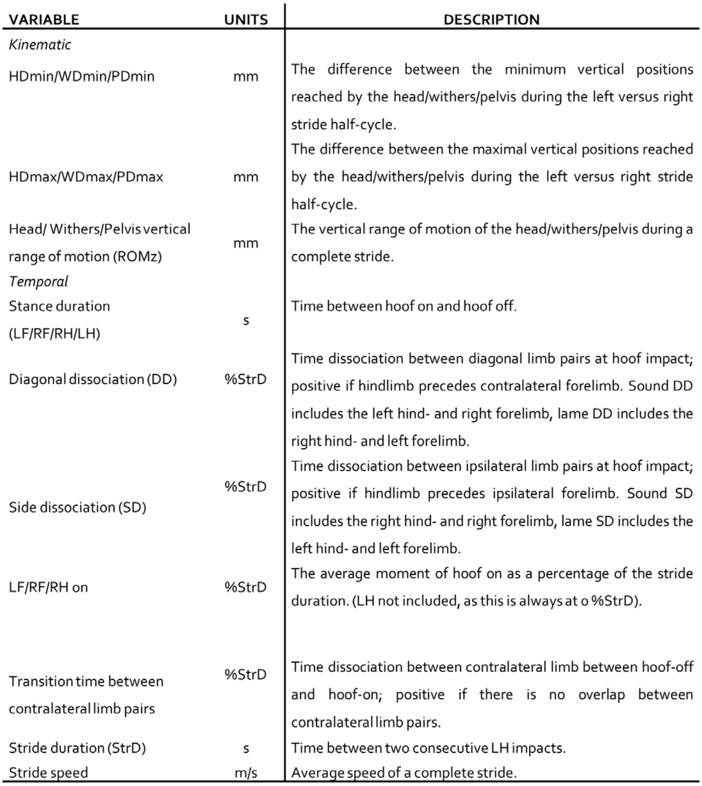


Figure 1. IMU node placement. Sensors attached at the following locations: in the median plane at the poll, wither and tubera sacrale; over each tuber coxae, on the lateral aspect of each mid cannon bone (in a protection pad) and on the lateral wall of each hoof. This image is from another study with a similar set up.[19] In the current study, riders were instructed to ride with loose rein contact, while this was not the case in the study depicted.

Kinematic data collection

The horses were equipped with 13 inertial measurement unit (IMU) nodes (of which 11 nodes were used for this study; Promove Mini, EquiMoves®)[18] set to a sampling frequency of 500 Hz, with the low-g accelerometer set at ±16 g and the high-g accelerometer set at ±200 g (Figure 1). In addition, the sacrum node was enabled with a global positioning system to measure the overground speed at 5 Hz. Data were stored using the internal memory of each node to ensure no data loss, and synchronisation between nodes was guaranteed with an error of less than 100 ns. All trials were video-recorded using standard equipment for retrospective analysis of the collected data.

A baseline measurement was performed on each horse before lameness induction. The baseline and induced lameness measurements included the following trials: walk and trot in-hand followed by walk, trot and tölt while ridden. Each horse's gaits were performed at a self-selected speed based on the rider's experience. For the ridden trials, the rider was instructed to keep light rein contact and perform sitting walk, trot and tölt not to affect the movement asymmetry by posting.[20] All trials for each horse were conducted on the same day and on the same hard surface (compacted lava gravel).



VARIABLE	UNITS	DESCRIPTION
Kinematic		
HDmin/WDmin/PDmin	mm	The difference between the minimum vertical positions reached by the head/withers/pelvis during the left versus right stride half-cycle.
HDmax/WDmax/PDmax	mm	The difference between the maximal vertical positions reached by the head/withers/pelvis during the left versus right stride half-cycle.
Head/ Withers/Pelvis vertical range of motion (ROMz)	mm	The vertical range of motion of the head/withers/pelvis during a complete stride.
Temporal		
Stance duration (LF/RF/RH/LH)	s	Time between hoof on and hoof off.
Diagonal dissociation (DD)	%StrD	Time dissociation between diagonal limb pairs at hoof impact; positive if hindlimb precedes contralateral forelimb. Sound DD includes the left hind- and right forelimb, lame DD includes the right hind- and left forelimb.
Side dissociation (SD)	%StrD	Time dissociation between ipsilateral limb pairs at hoof impact; positive if hindlimb precedes ipsilateral forelimb. Sound SD includes the right hind- and right forelimb, lame SD includes the left hind- and left forelimb.
LF/RF/RH on	%StrD	The average moment of hoof on as a percentage of the stride duration. (LH not included, as this is always at 0 %StrD).
Transition time between contralateral limb pairs	%StrD	Time dissociation between contralateral limb between hoof-off and hoof-on; positive if there is no overlap between contralateral limb pairs.
Stride duration (StrD)	s	Time between two consecutive LH impacts.
Stride speed	m/s	Average speed of a complete stride.

Table 1. Definitions of parameters and the units they are measured in.

Lameness induction

A well-established and fully reversible sole-pressure model was used to induce lameness.[21] Each horse was shod with a custom-made shoe with a mediolateral bar designed for applying pressure on the tip of the frog. A flat-edged bolt was inserted into the hole of the bar and tightened to apply the pressure. The lameness was randomly induced on the left or right forelimb by lottery. Once a mild lameness was deemed visible at the in-hand trot by the experienced veterinarians in the research team (E.H., M.R. and F.S.B.), the pressure was considered sufficient, and the measurement protocol was continued. Mild lameness was defined as a score of 1 to 2 out of five on an ordinal 0–5 degree lameness scale, where the two degrees of lameness were defined as follows: 1 subtle lameness: intermittently visible at the trot; 2 mild lameness: visible in every stride at the trot.[22]

Data analysis

Data analyses were performed in Matlab (version 2022b, MathWorks). All data (both baseline and induction) from horses with induced right forelimb lameness were mirrored by multiplying accelerometer data (both high and low; around the *y*-axis) and gyroscope (around the *x*- and *z*-axis) of all IMUs by -1. This way, all horses were analysed as being lame on the left forelimb to allow exploration of group-level lameness adaptation strategies. The trials were split into gait segments (walk, trot and tölt) based on a gait classification algorithm[19] and cut into strides based on the left hind (LH) impact. Hoof-on and hoof-off events for each limb were calculated based on manually labelled events from hoof-mounted IMU acceleration and gyroscope data. In short, a semi-supervised approach was used to detect stance and swing from each limb, using a time-series machine learning approach similar to a previously described algorithm.[23] A sequence-to-sequence regression approach using a long-term short-memory neural network was used, using the limb and hoof sensor data as input and the swing/stance phase of each limb as output. The moments of change between the swing and the stance phase were detected and classified as hoof-on and hoof-off moments.

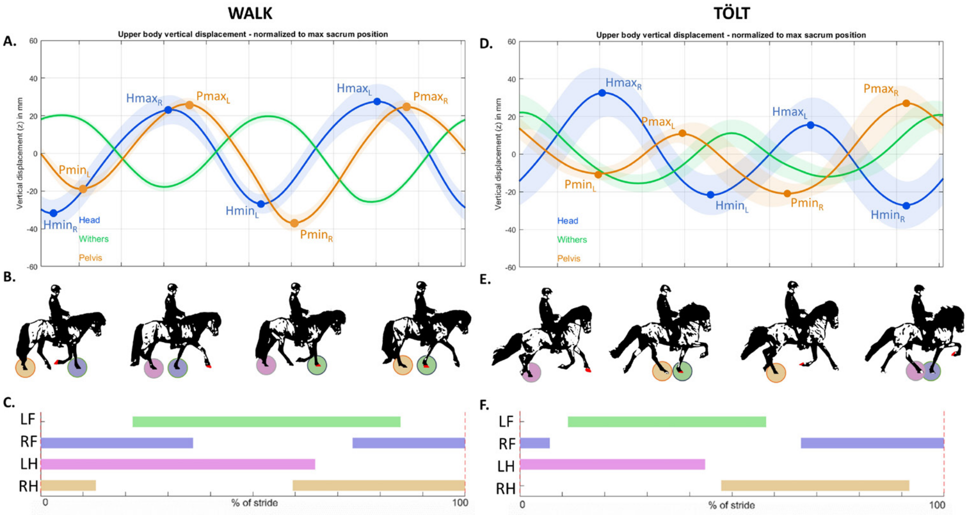


Figure 2. Explanations for upper body asymmetry value calculations. Upper body asymmetry values are calculated by subtracting left (L) step values from right (R) step values. This is done for the head (HDmin/HDmax), withers and pelvis (PDmin/PDmax) separately. For the head and withers, this means that the second peak or trough is subtracted from the first. This is reversed for the PDmin and PDmax calculations (second peak/trough minus the first peak/trough). Panels A–C show the example of a walk, whereas in Panels D–F, the tölt example is shown. Panels A and D show the upper body vertical movement of the head (blue), withers (green) and pelvis (orange). Panels B–F show a footfall pattern of the Icelandic horse at walk (B, C) tölt (E, F). In Panels B and E, the coloured circles indicate foot contact with the ground. In Panels C and F, the footfalls of the left front (LF; green), right front (RF; purple), left hind (LH; pink) and right hind (RH; orange) are shown over the mean stride (0%–100%). At the walk, negative values meant that a lower minimal and maximal position of the head and pelvis were reached during the right fore- and hindlimb stance phase, respectively. At the tölt, negative values meant that the head and withers reached a lower minimal position and the pelvis reached a lower maximal position during the stance phase of the left forelimb and that the head and withers reached a lower maximal position and the pelvis reached a lower minimal position during the stance phase of the left hindlimb.

Furthermore, for all tölt segments, individual strides were labelled as TT, tölt with lateral couplets (TLC) or mixed tölt (TMix; tölt with tripedal support), based on the side dissociation, ipsi- and diagonal support phases of the limbs.[1]

Primary outcome measures

Upper body asymmetry metrics and temporal stride parameters were extracted from each stride-segmented signal (Table 1). For the upper body asymmetry parameters, the stride split vertical displacement trajectories of the head (H), withers (W) and pelvis (P) were used. For all three locations, the difference between the vertical displacement minima (HD_{min}, WD_{min} and PD_{min}) and vertical displacement maxima(HD_{max}, WD_{max} and PD_{max}) were calculated (Figure 2 for the walk and tölt examples).

Except for stride speed and stride duration and stance durations of all four limbs, temporal stride parameters were calculated from the time-normalised stride data (LH impact to next LH impact = 0%–100% of stride duration [%StrD]). Footfalls of the individual limbs (left front [LF], right front [RF], LH and right hind [RH]) are displayed as %StrD, where LH impact is always at 0%StrD. Time differences between the footfalls within different limb pairs (diagonal and side dissociation) and transitions between contralateral limb pairs were also calculated from the time-normalised strides.

Statistical analysis

Linear mixed models were created for the two conditions (in-hand and ridden) separately, containing all gaits performed during that condition, to test the effect of lameness induction on both the kinematic and temporal stride parameters. In addition, linear mixed models were created for the ridden condition where the tölt strides were split into TT, TLC and TMix. Stride-level data for all variables were entered into the model from the baseline and induced lameness measurements. The models were built in R-studio (version 1.1.414, RStudio Inc.) using package nlme (version 3.1–152). In each model, the factor 'lameness' (baseline or induction) was entered as a fixed effect in interaction with 'gait' (walk, trot or tölt). Furthermore, 'horse' was entered as a random intercept. To correct for speed differences within

horses between baseline and induction and to improve model fit, 'stride speed' was used as a random slope. Correlations between different gaits (due to the non-random order of gaits within the horse) were estimated using an autocorrelation component in each model. To test for speed differences between baseline and induction trials within gait, a model was created where 'stride speed' was not included as a random slope. Model fit was evaluated using AIC values, q–q plots and residual plotting. Model estimates were represented as estimated marginal means, statistical significance was set at $\alpha = 0.05$, and *p* values were adjusted for multiple comparisons using the false discovery rate procedure.[24] To identify kinematic and temporal parameters that, on a group level, would be likely associated with lameness at the tölt, the sensitivity and specificity of the variables listed in Table 1 were calculated using package pROC (version 1.18).

For each horse, the 15 strides closest to the median stride were selected for both the baseline and induced conditions. Then, the sensitivity and specificity were calculated for each variable based on the difference between baseline and induction conditions. Finally, the optimal sensitivity, specificity, and corresponding threshold were calculated using Youden's index.

		WALK			TROT		
VARIABLE	**Units**	**Baseline**	**Induction**	**Difference**	**Baseline**	**Induction**	**Difference**
Kinematic							
HDmin	mm	1.1 [-4.8 - 7.0]	-17.7 [-23.8 - -11.6]	-18.8*	-7.2 [-14.3 - -0.1]	-25.3 [-32.7 - -18.0]	-18.14*
WDmin	mm	0.0 [-2.9 - 2.8]	0.2 [-2.8 - 3.3]	0.27	0.8 [-1.4 - 3.0]	-4.7 [-7.0 - -2.4]	-5.50*
PDmin	mm	-4.7 [-7.6 - -1.7]	0.1 [-3.2 - 3.4]	4.77*	0.4 [-2.7 - 3.4]	-3.8 [-6.9 - -0.6]	-4.13*
HDmax	mm	0.8 [-2.4 - 3.9]	6.5 [3.1 - 10.0]	5.75*	-8.1 [-12.7 - -3.5]	-15.9 [-20.8 - -11.0]	-7.81*
WDmax	mm	2.8 [1.1 - 4.5]	1.4 [-0.4 - 3.3]	-1.41*	-2 [-4.5 - 0.6]	-6.4 [-9.1 - -3.7]	-4.43*
PDmax	mm	-1.9 [-4.1 - 0.3]	-1.2 [-3.4 - 1.1]	0.74	-3.9 [-7.5 - -0.2]	-1.6 [-5.4 - 2.2]	2.29*
Head ROMz	mm	80.4 [72.0 - 88.8]	85.4 [76.7 - 94.1]	4.97*	59.1 [50.6 - 67.6]	68.3 [59.6 - 77.0]	9.20*
Withers ROMz	mm	42.2 [36.3 - 48.2]	45.6 [39.4 - 51.7]	3.33*	53.6 [48.1 - 59.0]	51.0 [45.5 - 56.5]	-2.59*
Pelvis ROMz	mm	69.6 [62.7 - 76.5]	69.2 [62.2 - 76.3]	-0.33	53.4 [46.7 - 60.0]	54.9 [48.2 - 61.5]	1.48
Temporal							
Stance duration LF	s	0.54 [0.53 - 0.56]	0.56 [0.54 - 0.58]	0.02*	0.25 [0.25 - 0.26]	0.28 [0.27 - 0.29]	0.03*
Stance duration RF	s	0.52 [0.50 - 0.54]	0.55 [0.53 - 0.58]	0.03*	0.24 [0.23 - 0.25]	0.26 [0.25 - 0.27]	0.02*
Stance duration LH	s	0.51 [0.49 - 0.53]	0.54 [0.51 - 0.56]	0.02*	0.23 [0.23 - 0.24]	0.24 [0.24 - 0.25]	0.01*
Stance duration RH	s	0.50 [0.47 - 0.52]	0.53 [0.50 - 0.55]	0.03*	0.24 [0.23 - 0.24]	0.25 [0.24 - 0.25]	0.01*
Lame diagonal dissociation	%StrD	27.1 [26.0 - 28.2]	26.9 [25.8 - 28.1]	-0.17	1.9 [0.9 - 2.9]	3.4 [2.4 - 4.4]	1.47*
Lame side dissociation	%StrD	22.9 [21.6 - 24.2]	22.6 [21.3 - 24.0]	-0.22	46.7 [45.5 - 47.9]	45.0 [43.8 - 46.2]	-1.69*
Sound diagonal dissociation	%StrD	27.5 [26.4 - 28.7]	27.8 [26.6 - 29.0]	0.27	4.1 [3.1 - 5.1]	5.9 [4.8 - 6.9]	1.77*
Sound side dissociation	%StrD	22.7 [21.8 - 23.6]	22.9 [22.0 - 23.8]	0.15	46.9 [46.2 - 47.7]	45.3 [44.5 - 46.1]	-1.60*
LF on	%StrD	22.9 [21.5 - 24.3]	22.8 [21.3 - 24.2]	-0.10	46.8 [45.5 - 48.0]	45.0 [43.7 - 46.3]	-1.76*
RF on	%StrD	-27.1 [-28.3 - -25.9]	-27.3 [-28.6 - -26.1]	-0.24	-4.2 [-5.4 - -3.1]	-6.3 [-7.4 - -5.2]	-2.03*
RH on	%StrD	49.6 [49.2 - 50.1]	49.3 [48.8 - 49.8]	-0.34*	49.1 [48.5 - 49.6]	48.9 [48.3 - 49.4]	-0.20
Transition RF to LF	%StrD	-12.8 [-13.5 - -12.2]	-13.0 [-13.7 - -12.3]	-0.19	5.3 [4.2 - 6.5]	3.8 [2.6 - 5.0]	-1.54*
Transition LF to RF	%StrD	-12.5 [-13.4 - -11.7]	-13.1 [-14 - -12.2]	-0.56	4.5 [3.3 - 5.6]	2.0 [0.9 - 3.2]	-2.44*
Transition RH to LH	%StrD	-12.4 [-13.4 - -11.4]	-12.1 [-13.1 - -11.1]	0.33	6.6 [5.3 - 7.8]	6.1 [4.8 - 7.4]	-0.43*
Transition LH to RH	%StrD	-12.3 [-12.7 - -11.9]	-12.7 [-13.2 - -12.3]	-0.44*	6.3 [5.3 - 7.3]	5.1 [4.0 - 6.1]	-1.25*

Results

A total of 10 horses were used for the analyses of the effects of lameness on temporal and kinematic gait parameters. For both in-hand and ridden conditions, speed did not differ between baseline and induction for any gait ($p > 0.05$; in hand: walk$_{\text{Baseline}}$/walk$_{\text{Induction}}$ 1.7/1.7 m/s and trot$_{\text{Baseline}}$/trot$_{\text{Induction}}$ 3.5/3.5 m/s; ridden: walk$_{\text{Baseline}}$/ walk$_{\text{Induction}}$ 1.5/1.5 m/s, trot$_{\text{Baseline}}$/ trot$_{\text{Induction}}$ 3.8/3.9 m/s and tölt$_{\text{Baseline}}$/tölt$_{\text{Induction}}$ 3.3/3.4 m/s). The ridden induction trial was lost for one horse due to missing data from one limb sensor. Therefore, the ridden data of this horse were excluded from further analyses.

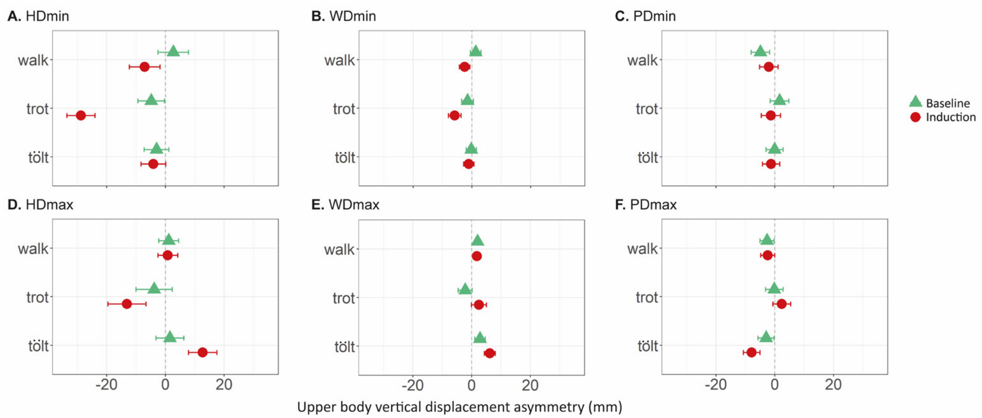


Figure 3. Graphical representation of linear mixed model results for upper body asymmetry during ridden trials. Linear mixed model results (estimated marginal means and confidence intervals) of the upper body symmetry values at the ridden walk, trot and tölt, before (green triangle) and after (red circle) lameness induction. Upper body asymmetry values of the head (H), withers (W) and pelvis (P) are given in mm. Both the difference between the two vertical displacement minima (HDmin, WDmin and PDmin) and between the vertical displacement maxima (HDmax, WDmax and PDmax) are shown. The data represent a group (n = 9) estimated marginal means (EMmeans) with the 95% confidence interval as crosshairs.

Kinematic parameters

Estimated marginal means for the kinematic parameters and their respective confidence intervals for each gait and condition can be found in Table 2 (in-hand trials) and Table 3 (ridden trials). The results for upper body vertical asymmetry parameters below are given in absolute difference between baseline and induction.

Upper body vertical asymmetry results of the ridden trials are shown in Figure 3. The HD$_{\text{min}}$ increased significantly ($p < 0.05$), both in-hand (18.8 and 18.1 mm) and ridden (9.7 and 23.9 mm; Figure 4A) for walk and trot, respectively, though remained unchanged during tölt. At the tölt, however, increases in HD$_{\text{max}}$ (11.2 mm; $p < 0.05$) and PD$_{\text{max}}$ (4.8 mm; $p < 0.05$) were found. Changes in HD$_{\text{max}}$ were found for both in-hand (7.8 mm; $p < 0.05$) and ridden (9.3 mm; $p < 0.05$) trot, where

Table 2. In hand walk and trot linear mixed models results. Note: Results are displayed as estimated marginal means [lower border confidence interval to upper border confidence interval]. The data represent group(n 10) estimated marginal means (EMmeans) with the = 95% confidence interval [lower border to upper border]. Abbreviations: LF, left forelimb; LH, left hindlimb; RF, right forelimb; RH, right hindlimb; ROMz, vertical range of motion; %StrD, percentage of the stride duration (calculated from LH-on to LH-on moments). *$p < 0.01$

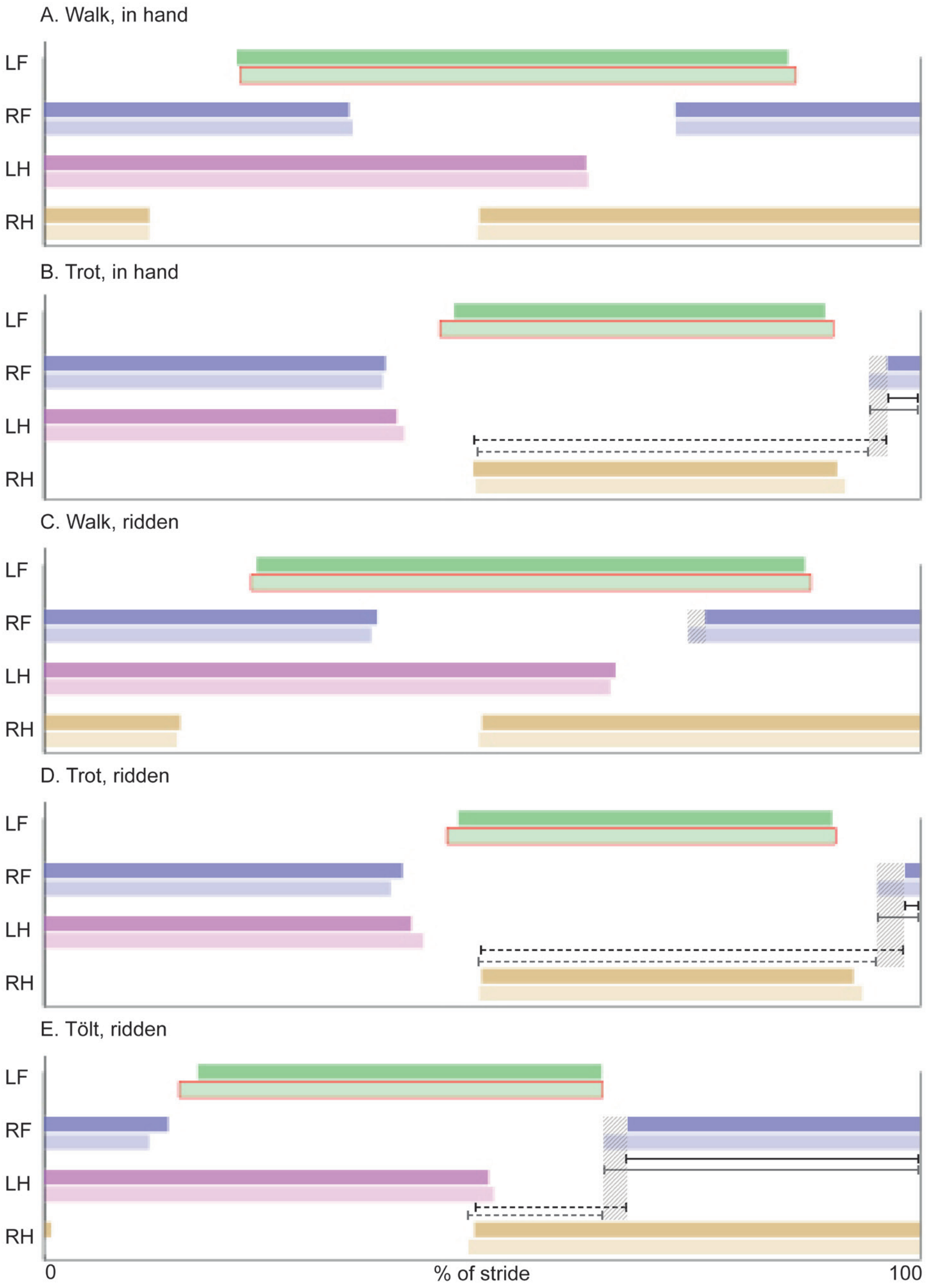


Figure 4. Graphical representation of the temporal changes. Footfall patterns during (A) walk in-hand, (B) trot in-hand, (C) ridden walk, (D) ridden trot and (E) ridden tolt, before (filled) and after (shaded) lameness induction of the left front (LF) limb. Significant changes in the footfall of the right front (RF) limb are displayed with the grey hatched area, which relates to the significant changes in diagonal dissociation (DD) (solid lines) and side dissociation (SD) (dashed lines). Black lines represent the baseline DD and SD, whereas grey lines represent DD and SD after induction. The data represents the group (n = 10 for in-hand trials and n = 9 for ridden trials) mean footfall patterns.

there was only a small change in PD_{max} while ridden (2.6 mm; $p < 0.05$). At the walk, HD_{max} only changed in-hand (5.8 mm; $p < 0.05$), and there was no change in PD_{max}. Furthermore, after lameness induction WD_{min} increased for in-hand (5.5 mm) and ridden (4.4 mm; $p < 0.05$) trot and ridden walk (3.8 mm; $p < 0.05$). WD_{max} changed with similar magnitude for both in-hand (4.4 mm; $p < 0.05$) and ridden (4.7 mm; $p < 0.05$) trot, though in-hand, the horses became more asymmetrical towards the lame limb, whereas while ridden, the asymmetry remained small but changed to the other side. At tölt, WD_{max} increased (3.4 mm; $p < 0.05$). During walk and trot, both in hand and ridden, the head's vertical range of motion (ROMz) increased after lameness induction, though more in-hand than ridden conditions. Withers ROMz increased in ridden walk (2.6 mm; $p < 0.05$) but decreased at ridden trot (4.4 mm; $p < 0.05$), and pelvis ROMz increased at ridden trot (2.9 mm; $p < 0.05$). At tölt, there were no changes before and after lameness induction in the head, withers and pelvis ROMz.

Temporal parameters

The effects of induced lameness on temporal stride parameters for each gait are summarised in Figure 4, and statistical results can be found in Table 2 for in-hand conditions and Table 3 for ridden conditions. At tölt, significant changes ($p < 0.05$) in temporal stride parameters include an increase in sound diagonal dissociation (2.2 %StrD) and a decrease in sound side dissociation (-1.4 %StrD), resulting in a lower percentage of TT strides after lameness induction (30.7% at baseline, 20.1% after induction). This is a result of an earlier landing of the sound forelimb (Figure 4) (-2.6 %StrD; $p < 0.05$), with no relevant changes in the footfall timings of the other limbs. Also, at the ridden walk (-1.7 %StrD; $p < 0.05$) and at in-hand trot (-2.0 %StrD; $p < 0.05$), the sound forelimb lands earlier relative to the stride cycle. This results in significant changes ($p < 0.05$) in sound diagonal dissociation (1.8 %StrD and 1.5 %StrD) and sound side dissociation (-1.6 %StrD and -1.5 %StrD) at in-hand and ridden trot, respectively, but not at walk. This earlier landing of the sound forelimb is also represented in the significant ($p < 0.05$) differences in the transition times from lame forelimb to sound forelimb, which decreases with -2.2 %StrD, -1.9 %StrD and -3.4 %StrD for ridden walk, trot and tölt, respectively, and with -0.6 %StrD and -2.4 %StrD for in-hand walk and trot.

Results of splitting the tölt

When comparing the effects of lameness between the different types of tölt, upper body vertical asymmetry for head, withers and pelvis changed the least for strides labelled as TT, except for HD_{min} (6.8 mm [$p < 0.05$], 0.5 mm and 0.2 mm [n.s.] for TT, TLC and TMix, respectively) and WD_{min} (4.3, 2.1 [$p < 0.05$] and 3.1 mm [n.s.] for TT, TLC and TMix, respectively). Changes were larger for TLC and TMix strides in HD_{max} (14.1 mm for TLC and 16.3 mm TMix; $p < 0.05$) and PD_{max} (8.1 mm for TLC and 8.6 mm for TMix; $p < 0.05$) compared to TT strides (7.3 mm for HD_{max} and 6.3 mm for PD_{max}; $p < 0.05$). The other effects of induced forelimb lameness during the different types of tölt (TT, TLC and TMix) are summarised in Table S1 and Figure S1.

		WALK			TROT			TÖLT		
VARIABLE	**Units**	**Baseline**	**Induction**	**Difference**	**Baseline**	**Induction**	**Difference**	**Baseline**	**Induction**	**Difference**
Kinematic										
HDmin	mm	2.7 [-2.5 - 7.9]	-7.1 [-12.3 - -1.9]	-9.74*	-4.8 [-9.4 - -0.3]	-28.8 [-33.5 - -24.0]	-23.92*	-3.0 [-7.2 - 1.2]	-4.1 [-8.3 - 0.1]	-1.09
WDmin	mm	1.3 [-0.5 - 3.1]	-2.5 [-4.3 - -0.7]	-3.76*	-1.5 [-3.5 - 0.5]	-5.9 [-8.0 - -3.7]	-4.37*	-0.2 [-2.0 - 1.5]	-1.1 [-2.9 - 0.7]	-0.85
PDmin	mm	-4.9 [-8.1 - -1.8]	-2.0 [-5.2 - 1.2]	2.91*	1.6 [-1.6 - 4.8]	-1.3 [-4.6 - 1.9]	-2.94*	-0.1 [-3.0 - 2.9]	-1.3 [-4.2 - 1.7]	-1.21
HDmax	mm	1.1 [-2.2 - 4.5]	0.8 [-2.5 - 4.2]	-0.31	-3.8 [-10.0 - 2.4]	-13.1 [-19.6 - -6.6]	-9.28*	1.5 [-3.2 - 6.3]	12.7 [7.9 - 17.6]	11.19*
WDmax	mm	2.0 [1.0 – 3.0]	1.8 [0.8 - 2.8]	-0.16	-2.2 [-4.6 - 0.2]	2.5 [0.0 – 5.0]	4.70*	2.8 [1.0 - 4.6]	6.2 [4.4 - 8.1]	3.37*
PDmax	mm	-2.7 [-5.0 - -0.3]	-2.4 [-4.8 – 0.0]	0.26	-0.2 [-3.2 - 2.8]	2.4 [-0.7 - 5.4]	2.56*	-3.0 [-5.7 - -0.2]	-7.9 [-10.7 - -5.0]	-4.88*
Head ROMz	mm	56.3 [48.7 - 63.8]	57.8 [50.2 - 65.4]	1.54	75.0 [67.1 - 82.9]	84.9 [76.8 - 93]	9.90*	77.9 [70.5 - 85.2]	75.4 [68 - 82.8]	-2.48
Withers ROMz	mm	21.4 [18.5 - 24.3]	24 [21.1 – 27.0]	2.63*	42.4 [39.5 - 45.2]	37.9 [35.0 - 40.9]	-4.43*	41.2 [38.5 - 43.8]	39.7 [37.1 - 42.4]	-1.43
Pelvis ROMz	mm	56.5 [51.6 - 61.3]	56.4 [51.5 - 61.2]	-0.11	48.0 [43.1 - 52.9]	50.9 [46.0 - 55.8]	2.86*	38.1 [33.3 - 42.9]	40.5 [35.7 - 45.3]	2.42*
Temporal										
Stance duration LF	s	0.54 [0.52 - 0.55]	0.56 [0.55 - 0.58]	0.03*	0.23 [0.22 - 0.24]	0.23 [0.22 - 0.24]	0.00	0.23 [0.22 - 0.24]	0.25 [0.24 - 0.26]	0.02*
Stance duration RF	s	0.51 [0.50 - 0.53]	0.54 [0.53 - 0.56]	0.03*	0.21 [0.20 - 0.22]	0.22 [0.20 - 0.23]	0.01	0.23 [0.22 - 0.24]	0.23 [0.22 - 0.24]	0.008*
Stance duration LH	s	0.55 [0.53 - 0.57]	0.58 [0.56 - 0.60]	0.03*	0.23 [0.22 - 0.24]	0.23 [0.23 - 0.24]	0.005	0.26 [0.25 - 0.27]	0.27 [0.26 - 0.28]	0.01
Stance duration RH	s	0.55 [0.53 - 0.57]	0.57 [0.55 - 0.59]	0.02*	0.22 [0.21 - 0.22]	0.22 [0.22 - 0.23]	0.009*	0.25 [0.24 - 0.26]	0.25 [0.24 - 0.26]	0.00
Lame diagonal dissociation	%StrD	26.1 [24.2 – 28.0]	26.1 [24.2 - 27.9]	-0.06	7.2 [3.8 - 10.6]	5.9 [3.3 - 8.5]	-1.37	31.4 [29.5 - 33.4]	31.9 [29.9 - 33.8]	0.41
Lame side dissociation	%StrD	23.3 [21.5 – 25.0]	22.7 [20.9 - 24.5]	-0.54	43.2 [40.8 - 45.7]	45.7 [43.3 - 48.2]	2.49*	17.8 [16.0 - 19.5]	16.8 [15.0 - 18.5]	-1.00*
Sound diagonal dissociation	%StrD	26.9 [25.5 - 28.2]	28.2 [26.8 - 29.5]	1.30*	7.4 [5.8 - 8.9]	5.8 [4.4 - 7.2]	-1.54*	33.2 [31.9 - 34.6]	35.4 [34.0 - 36.7]	2.17*
Sound side dissociation	%StrD	23.5 [21.9 - 25.2]	22.7 [21.1 - 24.3]	-0.85*	43.1 [41.3 - 44.9]	42.0 [40.3 - 43.8]	-1.05	17.4 [15.8 - 18.9]	15.9 [14.3 - 17.5]	-1.44*
LF on	%StrD	23.0 [21.2 - 24.7]	22.5 [20.8 - 24.3]	-0.42	45.2 [43.0 - 47.4]	46.6 [44.4 - 48.7]	1.38	18.0 [16.2 - 19.7]	16.9 [15.1 - 18.6]	-1.09*
RF on	%StrD	-26.5 [-27.8 - -25.3]	-28.2 [-29.5 - -27.0]	-1.71*	-6.1 [-7.3 - -4.8]	-5.5 [-6.7 - -4.2]	0.59	-33.1 [-34.3 - -31.8]	-35.6 [-36.9 - -34.4]	-2.58*
RH on	%StrD	50.0 [49.6 - 50.4]	49.4 [49.0 - 49.8]	-0.57*	50.7 [50.2 - 51.2]	50.6 [50.0 - 51.1]	-0.14	49.5 [49.1 - 50]	48.9 [48.4 - 49.4]	-0.64*
Transition RF to LF	%StrD	-13.2 [-14.6 - -11.8]	-13.2 [-14.6 - -11.8]	-0.01	2.9 [1.3 - 4.5]	2.8 [1.2 - 4.3]	-0.14	0.1 [-1.4 - 1.6]	1.0 [-0.5 - 2.5]	0.85*
Transition LF to RF	%StrD	-8.8 [-10.4 - -7.3]	-11.1 [-12.6 - -9.5]	-2.24*	4.4 [2.8 - 6]	2.5 [0.9 - 4.1]	-1.86*	1.8 [0.3 - 3.4]	-1.5 [-3.1 – 0.0]	-3.37*
Transition RH to LH	%StrD	-15.7 [-17.0 - -14.4]	-15.1 [-16.4 - -13.8]	0.58	0.1 [-1.3 - 1.6]	-1.6 [-3.0 - -0.1]	-1.68*	-6.6 [-8.0 - -5.2]	-5.9 [-7.2 - -4.5]	0.71
Transition LH to RH	%StrD	-12.9 [-14.2 - -11.6]	-12.9 [-14.1 - -11.6]	0.04	2.9 [1.6 - 4.2]	1.4 [0.1 - 2.7]	-1.48*	-5.2 [-6.5 - -4]	-6.8 [-8.0 - -5.5]	-1.53*

Sensitivity and specificity

The sensitivity and specificity results can be found in Table 4 and were also plotted in ROC curves (Figures S2–S4). For walk and trot, both in-hand and ridden, HD_{min} is the most sensitive and specific variable to discriminate between baseline and induction. WD_{min} seems to be a relevant variable only in in-hand (not ridden) trot (specificity = 0.77, sensitivity = 0.66). For tölt, kinematic variables have either low specificity, low sensitivity or both. For temporal variables, the transition time from LF to RF seems to be most sensitive for tölt and in-hand walk, whereas right-side dissociation seems to be most sensitive and specific for in-hand trot.

Discussion

This is the first study that describes the effect of induced forelimb lameness on the gait kinematics of Icelandic horses, presenting both upper body asymmetry and stride temporal parameters. Our results support our hypothesis that Icelandic horses show compensatory mechanisms to induced forelimb lameness, but these differ between the studied gaits. Both walk and trot, as well as tölt, were included in the study to better understand lameness adaptations in both walking and running gaits. This was particularly interesting since, based on empirical evidence, orthopaedic veterinarians often state that Icelandic horses can have problems performing trot during lameness exams. Other gaits, such as pace and canter, were not included in our study for safety reasons. Previous attempts to quantify lameness in canter have failed, mainly due to the canter being an asymmetrical gait, which does not allow for the calculation of asymmetry variables.[25]

As expected, at walk and trot, both in hand and ridden, vertical movement asymmetry of the head increased. The absolute magnitude of the head vertical movement asymmetry was larger at trot compared to walk, which is consistent with previous forelimb lameness studies in warmblood horses.[5] Furthermore, withers' vertical movement asymmetry (WD_{min}) changed in the same direction as HD_{min} at in-hand trot, which is consistent with findings in lame warmblood horses with forelimb lameness.[22] Based on a study by Pfau, the vertical movement asymmetries after lameness induction should be large enough to be detectable by veterinarians.[26] However, that study was performed using warmblood horses and needs to be repeated for smaller breeds such as the Icelandic horse. Nonetheless, Icelandic horses seem to adapt to forelimb lameness in a similar fashion as warmblood horses do at walk and trot in terms of upper body movement asymmetry.

The increase in vertical movement asymmetry of the head, indicating impact lameness (HD_{min}) at the trot and, to a lesser extent, at the walk, was not found at tölt. However, more systematic increases in asymmetry between the maximum positions of the head (HD_{max}) and the pelvis

◀ Table 3. Ridden walk, trot and tölt linear mixed models results. Note: Results are displayed as estimated marginal means [lower border confidence interval to upper border confidence interval]. The data represent group(n 10) estimated marginal means (EMmeans) with the = 95% confidence interval [lower border to upper border]. Abbreviations: LF, left forelimb; LH, left hindlimb; RF, right forelimb; RH, right hindlimb; ROMz, vertical range of motion; %StrD, percentage of the stride duration (calculated from LH-on to LH-on moments). $*p < 0.01$

		WALK IN-HAND			TROT IN-HAND			WALK RIDER			TROT RIDER			TÖLT RIDER		
VARIABLE	**Units**	**threshold**	**specificity**	**sensitivity**	**threshold**	**specificity**	**sensitivity**	**threshold**	**specificity**	**sensitivity**	**threshold**	**specificity**	**sensitivity**	**threshold**	**specificity**	**sensitivity**
Kinematic																
HDmin	mm	-7.32	0.74 [0.67 - 0.81]	0.71 [0.63 - 0.80]	-24.49	0.93 [0.89 - 0.97]	0.64 [0.56 - 0.73]	-0.24	0.57 [0.49 - 0.65]	0.65 [0.58 - 0.73]	-13.88	0.80 [0.72 - 0.87]	0.79 [0.71 - 0.87]	45.74	0.09 [0.05 - 0.14]	0.98 [0.96 - 1.0]
WDmin	mm	0.69	0.59 [0.50 - 0.67]	0.47 [0.38 - 0.56]	-4.15	0.77 [0.70 - 0.84]	0.66 [0.57 - 0.74]	-2.64	0.84 [0.77 - 0.91]	0.45 [0.37 - 0.54]	-8.09	0.85 [0.79 - 0.91]	0.43 [0.33 - 0.52]	-3.92	0.74 [0.66 - 0.82]	0.43 [0.35 - 0.52]
PDmin	mm	-0.56	0.57 [0.49 - 0.65]	0.58 [0.49 - 0.67]	-4.61	0.73 [0.65 - 0.80]	0.49 [0.40 - 0.58]	-0.21	0.71 [0.64 - 0.79]	0.49 [0.41 - 0.57]	-6.45	0.88 [0.82 - 0.94]	0.32 [0.24 - 0.41]	-11.03	0.12 [0.07 - 0.18]	0.93 [0.89 - 0.97]
HDmax	mm	21.01	0.93 [0.88 - 0.97]	0.19 [0.13 - 0.27]	-11.06	0.72 [0.64 - 0.80]	0.58 [0.48 - 0.67]	0.81	0.57 [0.48 - 0.65]	0.51 [0.44 - 0.59]	-24.94	0.86 [0.80 - 0.92]	0.38 [0.29 - 0.48]	-5.67	0.47 [0.39 - 0.56]	0.77 [0.71 - 0.85]
WDmax	mm	-3.55	0.84 [0.77 - 0.90]	0.29 [0.21 - 0.36]	-8.16	0.87 [0.81 - 0.92]	0.42 [0.33 - 0.51]	-0.32	0.48 [0.38 - 0.57]	0.66 [0.58 - 0.74]	-2.99	0.48 [0.39 - 0.58]	0.78 [0.69 - 0.86]	9.28	0.72 [0.65 - 0.80]	0.49 [0.40 - 0.57]
PDmax	mm	-3.48	0.67 [0.60 - 0.75]	0.39 [0.31 - 0.48]	10.12	0.93 [0.89 - 0.97]	0.33 [0.24 - 0.41]	-0.09	0.75 [0.68 - 0.82]	0.40 [0.32 - 0.48]	2.60	0.76 [0.69 - 0.84]	0.51 [0.42 - 0.60]	3.91	0.26 [0.19 - 0.34]	0.87 [0.81 - 0.92]

		WALK IN-HAND			TROT IN-HAND			WALK RIDER			TROT RIDER			TÖLT RIDER		
VARIABLE	**Units**	**threshold**	**specificity**	**sensitivity**	**threshold**	**specificity**	**sensitivity**	**threshold**	**specificity**	**sensitivity**	**threshold**	**specificity**	**sensitivity**	**threshold**	**specificity**	**sensitivity**
Temporal																
Lame diagonal dissociation	s	25.78	0.84 [0.78 - 0.90]	0.36 [0.28 - 0.46]	2.88	0.65 [0.57 - 0.73]	0.74 [0.66 - 0.82]	26.92	0.64 [0.56 - 0.72]	0.49 [0.40 - 0.56]	4.62	0.79 [0.71 - 0.87]	0.36 [0.26 - 0.45]	31.96	0.55 [0.46 - 0.63]	0.74 [0.66 - 0.81]
Lame side dissociation	s	24.72	0.93 [0.87 - 0.96]	0.21 [0.13 - 0.28]	46.04	0.67 [0.59 - 0.74]	0.69 [0.60 - 0.77]	26.23	0.30 [0.22 - 0.38]	0.86 [0.80 - 0.91]	45.04	0.82 [0.75 - 0.88]	0.36 [0.28 - 0.46]	14.64	0.82 [0.76 - 0.89]	0.47 [0.39 - 0.56]
Sound diagonal dissociation	s	24.82	0.91 [0.86 - 0.95]	0.18 [0.11 - 0.25]	4.51	0.68 [0.60 - 0.76]	0.75 [0.67 - 0.83]	25.42	0.50 [0.41 - 0.59]	0.73 [0.66 - 0.80]	5.82	0.90 [0.84 - 0.95]	0.40 [0.31 - 0.50]	37.10	0.87 [0.81 - 0.92]	0.36 [0.28 - 0.44]
Sound side dissociation	s	23.49	0.75 [0.67 - 0.82]	0.41 [0.33 - 0.50]	46.96	0.57 [0.49 - 0.65]	0.95 [0.91 - 0.98]	26.35	0.39 [0.30 - 0.47]	0.81 [0.75 - 0.87]	43.80	0.95 [0.91 - 0.98]	0.31 [0.23 - 0.40]	16.22	0.64 [0.56 - 0.72]	0.67 [0.59 - 0.75]
LF on	%StrD	48.65	0.85 [0.79 - 0.91]	0.36 [0.27 - 0.44]	50.33	0.87 [0.81 - 0.93]	0.30 [0.22 - 0.38]	49.36	0.66 [0.58 - 0.74]	0.52 [0.44 - 0.61]	48.95	0.78 [0.70 - 0.85]	0.39 [0.30 - 0.49]	48.97	0.56 [0.48 - 0.65]	0.49 [0.41 - 0.58]
RF on	%StrD	24.89	0.93 [0.89 - 0.97]	0.22 [0.15 - 0.30]	45.88	0.70 [0.63 - 0.78]	0.65 [0.56 - 0.74]	23.54	0.55 [0.46 - 0.63]	0.61 [0.53 - 0.69]	45.04	0.8 [0.72 - 0.87]	0.34 [0.25 - 0.43]	14.56	0.81 [0.74 - 0.87]	0.47 [0.38 - 0.55]
RH on	%StrD	-26.94	0.72 [0.63 - 0.79]	0.37 [0.29 - 0.46]	-4.58	0.69 [0.60 - 0.76]	0.75 [0.68 - 0.83]	-25.82	0.57 [0.48 - 0.66]	0.68 [0.60 - 0.75]	-6.35	0.96 [0.91 - 0.99]	0.32 [0.23 - 0.41]	-33.84	0.54 [0.45 - 0.62]	0.68 [0.59 - 0.75]
Transition RF to LF	%StrD	-13.99	0.76 [0.68 - 0.83]	0.33 [0.25 - 0.42]	8.74	0.48 [0.40 - 0.57]	0.80 [0.72 - 0.87]	-13.69	0.67 [0.59 - 0.76]	0.49 [0.40 - 0.57]	7.75	0.45 [0.35 - 0.54]	0.70 [0.60 - 0.80]	3.99	0.60 [0.52 - 0.67]	0.82 [0.76 - 0.88]
Transition LF to RF	%StrD	-11.30	0.31 [0.23 - 0.40]	0.88 [0.82 - 0.94]	5.56	0.77 [0.69 - 0.84]	0.65 [0.57 - 0.74]	-11.04	0.42 [0.33 - 0.50]	0.87 [0.81 - 0.92]	3.72	0.96 [0.93 - 0.99]	0.46 [0.37 - 0.56]	2.53	0.56 [0.47 - 0.65]	0.91 [0.86 - 0.95]
Transition RH to LH	%StrD	-10.76	0.90 [0.85 - 0.95]	0.28 [0.20 - 0.36]	10.07	0.53 [0.45 - 0.62]	0.73 [0.65 - 0.81]	-16.28	0.40 [0.31 - 0.49]	0.80 [0.73 - 0.86]	7.98	0.60 [0.51 - 0.69]	0.67 [0.57 - 0.76]	2.30	0.25 [0.17 - 0.33]	0.96 [0.93 - 0.99]
Transition LH to RH	%StrD	-13.27	0.78 [0.71 - 0.85]	0.41 [0.32 - 0.50]	8.88	0.55 [0.47 - 0.64]	0.58 [0.48 - 0.65]	-15.23	0.52 [0.43 - 0.61]	0.60 [0.52 - 0.68]	6.44	0.85 [0.77 - 0.91]	0.57 [0.47 - 0.66]	-0.85	0.43 [0.34 - 0.52]	0.94 [0.90 - 0.98]

Table 4. Receiver operating characteristic (ROC) analyses results for discrimination between sound and lame. Note: The threshold with corresponding specificity and sensitivity are given for all variables, both kinematic and temporal for in-hand (A) and ridden (B) trials. The Youden index method was used and the values represent the ʻʻ optimal value to discriminate between baseline and induction. The data represent group (n 10 for in-hand trials and n 9 for ridden trials) point estimates with their corresponding 95% confidence interval [lower border to == upper border]. Abbreviations: LF, left forelimb; LH, left hindlimb; RF, right forelimb; RH, right hindlimb; %StrD, percentage of the stride duration (calculated from LH-on to LH-on moments)

(PD_{max}) were found, although some horses showed similar values for HD_{max} at baseline. The pattern in vertical displacement maxima differences at tölt seems similar, though opposite and with smaller changes, to the pattern in vertical displacement minima differences after forelimb lameness induction at trot.[22] That is to say, the head and withers asymmetry are pointing towards the same side, whereas the pelvis asymmetry points towards the opposite side. Following lameness induction, a lower maximum head position was reached when the lame limb pushed off from the ground. The lower maximum position of the pelvis occurs when the lame forelimb is in midstance and the ipsilateral hindlimb pushes off. This could indicate that the horse is pitching its body backwards, trying to use the pelvis and back to decrease the loading of the lame forelimb. The increase in pelvis vertical movement asymmetry may confuse the observer in determining the affected limb, as asymmetries of the pelvis are often related to hindlimb lameness.[6,8,26]

Some apparent adaptations to lameness at tölt, and to a lesser extent at walk and trot, seem to occur in the footfall pattern. Our results show that the Icelandic horse tries to decrease the loading of the lame forelimb by advancing the placement (i.e., the earlier timing of the hoof impact) of the sound forelimb. This earlier placement ultimately decreases the transition time from the lame to the sound forelimb, whereas the transition time from the sound to the lame forelimb remains unaffected. This might be caused by lower peak vertical forces and impulses produced by the lame forelimb.[6,7,10,21] Similar adaptations have been found in warmblood horses with forelimb lameness at trot, where the suspension from the lame to the sound diagonal was 61% shorter compared to baseline and not even half as long as the suspension from the sound to the lame diagonal.[27] Due to the earlier landing of the sound forelimb, the same asymmetry translates into the differences in diagonal dissociation and side dissociation between the sound and the lame side. At trot and tölt, right diagonal dissociation and right-side dissociation, which include the non-lame forelimb, change, while left diagonal dissociation and left side dissociation, which include the lame forelimb, remain unaffected.

The stance duration of both forelimbs increased during all gaits and conditions except for the ridden trot. The increase in stance duration has been described in trot as one of the primary mechanisms horses use to reduce peak GRFz of the lame limb.[21] As tölt is also considered a running gait, it was not surprising to find increases in stance duration of the lame forelimb after lameness induction in this gait. However, a recent study showed a reduction in stance duration of the lame limb at walk,[10] which contrasts with our results. Differences between treadmill and overground locomotion may cause this discrepancy, as it is known that in trot, the stance duration of the forelimbs is higher on the treadmill compared to overground conditions.[28] However, whether this holds true for walk is unknown. Another cause may be that speed could not be perfectly matched between baseline and induction trials in our study. Even though on average speed did not change after lameness induction and stride speed was corrected for in the statistical models, the horses could have compensated by slightly changing the speed between trials. Finally, the increase in stance duration at the walk is likely not a rider effect since stance duration at the walk after lameness induction increased with similar magnitude during both in-hand and ridden conditions.

The increase in upper body vertical movement asymmetry was more considerable during the in-hand conditions compared to ridden conditions, even though the rider was asked to perform a sitting trot and only have light rein contact to reduce the rider's influence in the head movement. It is known that, by posting, riders can increase or decrease vertical movement asymmetry.[20,29] However, the rider was not expected to affect the movement asymmetry in our study, as the rider stayed seated during all gaits to avoid asymmetrical loading.[30,31]

The horses used for this study were judged as non-lame before inclusion. This soundness was further confirmed by the absence of significant asymmetries at both in-hand and ridden walk and trot during the baseline measurements. Interestingly, substantial upper body vertical movement asymmetries were found in the baseline tölt trials. A possible explanation is that the tölt might be a more complex task for the horse than to walk or to trot. It can be found in the literature that elite dressage horses are more symmetrical at trot compared to passage or piaffe in terms of the vertical centre of mass movement.[32] Furthermore, it has been shown from human studies that healthy young adults move more asymmetrically in terms of limb kinematics and vertical centre of mass movement when dual tasks need to be performed, thus when a task has increased complexity.[33] The increased complexity theory is supported by the notice that Icelandic horses, unless extremely good tölters, do not perform a pure tölt unless ridden, for instance, when they are out in the field or run in- hand. These baseline asymmetries at tölt in sound Icelandic horses possibly make it more challenging to examine a horse for lameness and to distinguish between physiological and pain-related movement asymmetries.

Studies have shown that lameness is easier to quantify at a trot compared to walk in warmblood horses.[10,34] It is suggested that this can be attributed to higher speed and the two-beat nature of the trot, which ultimately increases peak GRFz, resulting in higher levels of discomfort when the lame limb is on the ground. Also, the centre of mass moves more vertically in trot when compared to walk, and this higher vertical centre of mass movement contributes to the higher peak vertical forces on the limbs.[10] However, with increasing speed, stride frequency increases, resulting in lower visibility of the asymmetries that might be present.[2] Like trot, tölt also has higher stride speed and frequency compared to walk[1,3,11,12,17]; therefore, higher peak GRFz can be expected. Following this reasoning, we expected to find increased upper body movement asymmetries after lameness induction at tölt. However, this was not found in the same manner as at the walk and trot. In contrast, similar to walk, the tölt has no suspension phase, and for the largest part of the stride cycle, at least two limbs are in contact with the ground. This might make it easier to redistribute weight away from the lame limb. Moreover, Polet showed that the vertical centre of mass motion should decrease with more distributed footfalls during the gait cycle.[16] This might explain why differences in HD_{min} and WD_{min} were not expressed at tölt compared to trot. Although it is still counterintuitive that substantial changes in these parameters are found at walk, but not at tölt after induction.

Since the horses in this study were only measured at tölt while being ridden, we could not discriminate between the effects of lameness and the possible confounding effects between the rider and lameness on the measured parameters at tölt. Substantial differences were observed between in-hand and ridden walk and trot, where movement asymmetries after lameness induction were smaller in ridden conditions. Some orthopaedic veterinarians mention that when Icelandic horses are presented for lameness exams, it is difficult to get them to or keep them in, trot. As a result, they may often show a tölt mixture or a pacy trot. We found that adaptations to lameness may differ between different 'types' of tölt, where the asymmetries were most prominent in this so-called TMix. Movement asymmetries could have been more pronounced if the horses had been tölted in hand, even though the quality of this tölt may have been low. Further research needs to be done with clinical cases to confirm this.

There were some limitations to this study. The first limitation is that we used an acute but reversible sole-pressure lameness induction model, which differs from many pathologies in lame horses. However, when the same lameness induction method on warmbloods was used,[22] lameness patterns were similar to those seen later in clinical cases with different kinds of pathologies.[35] Second, only one surface type was investigated, which is known to affect kinematics and stride temporal parameters.[36] Finally, only subtle/mild lameness was induced to minimise the discomfort experienced by the horses during this study. Therefore, we could not investigate if different degrees of lameness result in different adaptation strategies.

Understanding the compensation mechanisms for lameness across gaits in the Icelandic horse is essential for a proper lameness diagnosis. Compensatory mechanisms are complex and demonstrate that, in general, kinematic adaptations at tölt are different from those at walk and trot. Furthermore, adaptations seem larger in in-hand compared to ridden conditions. At tölt, as opposed to walk and trot, asymmetry in the vertical movement minima of the head appears less indicative of forelimb lameness, making visual assessment more challenging. Based on the results of this study, it is advised to primarily use the walk and trot for lameness assessments in the Icelandic horse. If a horse is unable to perform trot in-hand, we advise performing the lameness examination while ridden at trot, with the rider performing sitting trot or standing in the stirrups.

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4

Adaptation strategies of the Icelandic horse with induced hindlimb lameness at walk, trot and tölt.

Manuscript in preparation

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Abstract

The Icelandic horse is well-liked for its smooth gait, but assessing lameness in this breed can be challenging. Vertical movement asymmetry of the minimum/maximum pelvis position is used to quantify weight-bearing (PDmin) and push-off (PDmax) lameness during trot, but there are no established parameters to detect hindlimb lameness in other gaits. To address this gap, this study focused on evaluating limb kinematics and upper-body movement symmetry when hindlimb lameness was induced, at various gaits.

Eleven clinically sound Icelandic horses were measured before and after hindlimb lameness induction. Data were measured at ridden walk, trot and tölt, and in-hand walk and trot, using an IMU-based system (500 Hz). Limb kinematics and HDmin/HDmax/PDmin/PDmax and Hip-Hike were assessed using linear mixed models ($P<0.05$), to compare between sound and lame conditions. Results are presented as estimated marginal means.

For all gaits, except walk in-hand, lameness induction significantly increased PDmin at walk ($\text{PDmin}_{\text{ridden}}$ 0.6 to -4.5mm), trot ($\text{PDmin}_{\text{hand}}$ -0.5 to –14.7mm, $\text{PDmin}_{\text{ridden}}$ -0.7 to –10.5mm), tölt, ($\text{PDmin}_{\text{ridden-}}$ 0.2 to –4.7mm). However, PDmax only increased significantly in trot ($\text{PDmax}_{\text{hand}}$ -3.4 to –8.2mm). Hip-hike also significantly increased at trot (hip hike $\text{swing}_{\text{hand}}$ –5.5 to –26.41mm, hip hike $\text{stance}_{\text{hand}}$ -3.4 to –25.1mm, Hip hike $\text{swing}_{\text{ridden}}$ –0.5 to –10.7mm, hip hike $\text{stance}_{\text{ridden}}$ 1.8 to –8.2mm) and tölt (hip $\text{hikeswing}_{\text{ridden}}$ –1.8 to –6.1mm, hip hike $\text{stance}_{\text{ridden}}$ –5.4 to –11.0 mm). PDmin and hip hike appear to be effective parameters to quantify hindlimb lameness in Icelandic horses when trotting both in-hand and while ridden. When ridden at walk, only slight alterations were noticed in PDmin. This might be attributed to the variances in single or bipedal hindlimb support, as the pelvic minimum position signifies distinct loading stages throughout the stride cycle for various gaits.

Introduction

Orthopedic disorders are one of the primary reasons for veterinary consultation in horses, and are diagnosed by veterinarians during lameness exams. Subjective lameness assessment is standard practice but it has proven to be challenging in trotting horses, reflected by the low inter-rater agreement among veterinarians evaluating lameness.[1,2] Assessment of hindlimb lameness has been shown to be more difficult compared to forelimb lameness.[1,3] The reason could be a smaller vertical movement amplitude and movement asymmetry of the pelvis compared to the head in lame horses[4,5], but this needs further investigation.

The Icelandic horse is popular due to its ability to perform tölt and pace, but lameness evaluation in this breed is experienced as challenging due to the higher stride frequency and lower pelvic range of motion compared to other breeds.[6] Their genetic predisposition for altered limb timing[7] may also influence how they compensate for lameness. This potentially makes lameness assessment in this breed more challenging, as the trot could shift into a three or four-beat gait with a lack of suspension.

Objective lameness assessment tools are commonly used in equine practice but these techniques are only validated for hindlimb lameness quantification at trot. So far, no quantification methods have been established for any of the remaining gaits. Vertical movement asymmetry of the minimum/maximum pelvis position is used to quantify hindlimb weight-bearing (PDmin) and push-off (PDmax) lameness at trot[8], but it is unknown if these variables are useful for lameness quantification in other symmetrical gaits. In contrast to trot, during walk the pelvis reaches the highest position during midstance and the lowest position during bilateral hindlimb stance. At walk, Buchner et al. found a small reduction in the tuber sacrale amplitude during the lame stance phase from 5.7 cm without lameness, to 5.1 cm in horses with induced hindlimb lameness.[4] It is unclear, however, when during the stance phase the reduction was observed, and how the vertical displacement relates to the uni- and bipedal hindlimb support.

Tölt is a four-beat, lateral sequence, ambling gait without suspension. At the tölt, the horse is alternating between bipedal and unipedal limb support, where eight different limb support phases can be distinguished for each stride. Ideally, 25% of the stride duration should elapse between subsequent hoof placements.[9] Similar to other running gaits, the tölt can be described using spring-mass mechanics. However, the footfall pattern and small vertical excursions of the center of mass, are typical of a walking gait.[10,11] The timings of the minima and maxima of head and withers at tölt are synchronized as in trot. While on the other hand, pelvis minima and maxima are around 25% of the stride duration out of phase with the head and withers, which is similar to walk.[6]

Compensatory ipsilateral head and contralateral withers movement asymmetry are often observed in hindlimb lame horses at trot. This can be attributed to the horse shifting its center of mass forward during diagonal limb support.[12,13] However, compensatory patterns in other gaits than trot remain unknown. It is suggested that Icelandic horses may be less effective in redistributing the loading between limbs at tölt compared to walk and trot as there are periods of unipedal support for each limb, including the lame limb.[14]

The understanding of lameness compensation strategies in Icelandic horses performing different gaits is a prerequisite for the development of algorithms for objective lameness quantification. Objective tools for lameness assessment will benefit from this understanding, which could increase the accuracy of lameness assessment in this breed. Therefore, this study aimed to evaluate limb kinematics and upper-body movement symmetry in response to induced hindlimb lameness in Icelandic horses during in-hand walk and trot, and ridden walk, trot and tölt. We hypothesized that Icelandic horses will show similar upper-body compensatory mechanisms to hindlimb lameness compared to warmblood horses at trot, though they are also expected to alter inter-limb timing. It is expected that these adaptations differ between walk, trot and tölt.

Material and methods

Horses

Eleven clinically sound Icelandic horses (age range 5 – 26 years) were included in the study. The horses were used for education at Hólar University, in full training and were able to perform walk, trot and tölt (eight with the homozygous AA genotype of the DMRT3 gene and three with heterozygote AC). Before the study, horses underwent a clinical examination including visual and objective lameness measurements at trot in-hand (Equinosis, St Louis, Missouri, USA), as well as clinical examinations of the locomotor apparatus by an experienced orthopedic veterinarian (MR, EH and FSB). The study was approved by the Icelandic Food and Veterinary Authority MAST (approval number: 2020-02-12). Informed consent for data collection was obtained from the horse owners prior to the study.

Kinematic data collection

Thirteen inertial measurement units (IMUs; Promove Mini, Inertia Technology) with a sampling frequency of 500 Hz, with the low-g accelerometer set at ± 16 g and the high-g accelerometer set at ± 200 g were used for motion analysis. The sensors were attached to the horse and data from eleven of the sensors were analyzed in the present study. The location of the sensors are illustrated in figure 1. The IMU on the sacrum included a global positioning system, to measure the overground speed at a sampling frequency of 5 Hz. An internal memory card of each sensor stored the data to ensure no data loss, and synchronization between nodes was guaranteed with an error of less than 100 ns. All trials were video recorded from the back and side views of the horse, using standard equipment for retrospective quality check of the measurements.

A baseline measurement was performed on each horse before lameness induction in walk and trot in-hand, followed by walk, trot and tölt while ridden. Pace and canter were omitted as canter is an asymmetric gait with limited value for lameness assessment[15], for safety reasons for both rider and horse, and because pace is not commonly assessed during lameness examinations. All measurements were performed on a straight line and on a hard surface (compact lava gravel) on the same day. The handler and rider determined a comfortable speed for each horse, and the rider was instructed to maintain a light rein contact and perform a seated walk, trot and tölt to not influence the head movement or induce pelvic movement asymmetries.[16]

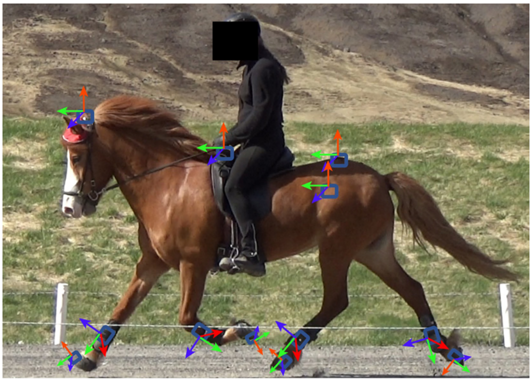

Figure 1. IMU node placement. Sensors attached at the following locations: in the median plane at the poll, wither and tubera sacrale; over each tuber coxae, on the lateral aspect of each mid cannon bone (in a protection pad) and on the lateral wall of each hoof. This image is from another study with a similar set up.19 In the current study, riders were instructed to ride with loose rein contact, while this was not the case in the study depicted.

Lameness induction

A well-established and fully reversible sole pressure model was used to induce lameness.[17] Each horse was shod with a custom-made shoe with a mediolateral bar designed to apply pressure on the tip of the frog. A flat-edged bolt was inserted into the hole of the bar and tightened until a mild lameness was visible at in-hand trot by the experienced veterinarians in the research team (MR, EH, HG, FSB). Mild lameness was defined as a score of 1 to 2 out of five on an ordinal 0-5 degree lameness scale, where the two degrees of lameness were defined as follows: 1 subtle lameness: irregularity not visible on every stride at the trot; 2 mild lameness: visible on every stride at the trot.[13] The lameness was randomly induced on the left or right hindlimb by lottery.

Data analysis

Data analyses were performed in Matlab (version 2022b, MathWorks, Natick, Massachusetts, USA) and has previously been described by Smit et al. 2023[18]. All data (both baseline and induction) from horses with induced right hindlimb lameness were mirrored by multiplying accelerometer (both high and low; around the y axis) and gyroscope (around the x and z axis) data of all IMUs by -1.

To explore group level lameness adaptation strategies, all horses were analyzed as being lame on the left hindlimb. Based on a gait classification algorithm[5], the trials were divided into gait segments (walk, trot, and tölt), and the strides were calculated based on the left hind (LH) impact. Hoof-mounted IMU acceleration and gyroscope data were used to determine the hoof-on and hoof-off events for each leg. To put it briefly, a time-series machine learning technique, comparable to the previously published algorithm[5], was utilized to determine stance and swing from each limb. Using the input from the limb and hoof sensor data and the long-term short memory (LSTM) network, a sequence-to-sequence regression approach was applied.

Additionally, based on the side dissociation, ipsi-, and diagonal support phases of the limbs, individual strides for each tölt segment were classified as true tölt (TT), tölt with lateral couplets (TLC), or mixed tölt (TMix; tölt with tripedal support).[19]

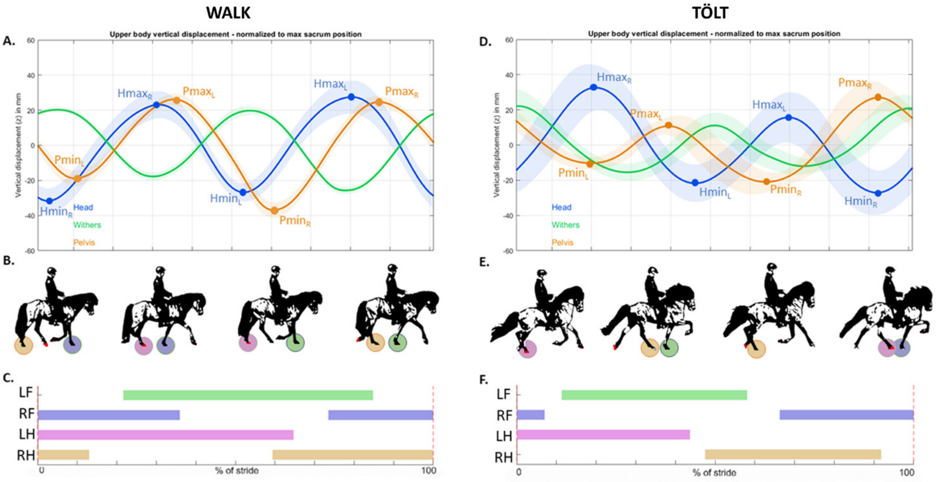

Figure 2 Explanations for upper body asymmetry value calculations. Upper body asymmetry values are calculated by subtracting left (L) step values from right (R) step values. This is done for the head (HDmin/HDmax), withers and pelvis (PDmin/PDmax) separately. For the head and withers, this means that the second peak or through is subtracted from the first. This is reversed for the PDmin and PDmax calculations (second peak/through minus the first peak/through). Panel A-C show the example for walk, whereas in panel D-F the tölt example is shown. Panel A and D show the upper body vertical movement of the head (blue), withers (green) and pelvis (orange). Panel B-F show a footfall pattern of the Icelandic horse at walk (B, C) tölt (E, F). In panel B and E the colored circles indicate foot contact with the ground. In panel C and F, the footfalls of the left front (LF; green), right front (RF; purple), left hind (LH; pink) and right hind (RH; orange) are shown over the mean stride (0-100%). At the walk (A-C), a negative value for the HDmin means that a higher minimal position of the head was reached during the left fore midstance compared to right fore midstance. For HDmax, a negative value means that a higher maximal position of the head is reached when the left forelimb is retracted and the right forelimb is protracted during double forelimb stance. For PDmin, a negative value means that a higher minimal position of the pelvis is reached when the left hindlimb is protracted and the right hindlimb is retracted at the end of stance phase. For PDmax, a negative value means that a higher maximal position of the pelvis is reached when the right hindlimb is at midstance and the left hindlimb is in swing. At the tölt (D-F), negative values mean that the head and withers reach a lower minimal position and the pelvis reached a lower maximal position during the stance phase of the left forelimb and that the head and withers reach a lower maximal position and the pelvis reached a lower minimal position during the stance phase of the left hindlimb.

VARIABLE	UNITS	DESCRIPTION
Kinematic		
HDmin/WDmin/PDmin	mm	The difference between the minimum vertical positions reached by the head/withers/pelvis during the left versus right stride half-cycle.
HDmax/WDmax/PDmax	mm	The difference between the maximal vertical positions reached by the head/withers/pelvis during the left versus right stride half-cycle.
Head/ Withers/Pelvis vertical range of motion (ROMz)	mm	The vertical range of motion of the head/withers/pelvis during a complete stride.
Hip hike stance/swing	mm	The difference in vertical displacement of the left and right tuber coxae during the stance and swing phase of the left and right hindlimb.
Temporal		
Stance duration (LF/RF/RH/LH)	s	Time between hoof on and hoof off.
Diagonal dissociation (DD)	%StrD	Time dissociation between diagonal limb pairs at hoof impact; positive if hindlimb precedes contralateral forelimb. Sound DD includes the left hind- and right forelimb, lame DD includes the right hind- and left forelimb.
Side dissociation (SD)	%StrD	Time dissociation between ipsilateral limb pairs at hoof impact; positive if hindlimb precedes ipsilateral forelimb. Sound SD includes the right hind- and right forelimb, lame SD includes the left hind- and left forelimb.
LF/RF/RH on	%StrD	The average moment of hoof on as a percentage of the stride duration. (LH not included, as this is always at 0 %StrD).
Transition time between contralateral limb pairs	%StrD	Time dissociation between contralateral limbs. At the walk the values display the time from hoof-on to hoof-off = double limb support in the front / hind pairs, at the tölt and trot the values display the time from hoof-off to hoof-on, i.e. the suspension for both fore or hind hooves (the time both are in the air).
Stride duration (StrD)	s	Time between two consecutive LH impacts.
Stride speed	m/s	Average speed of a complete stride.

Table 1. Definitions of tested parameters and the units they are measured in. Abbreviations: LF: left forelimb, RF: right forelimb, RH: right hindlimb, LH: left hindlimb, mm: millimeter, %StrD: percentage of the stride duration, s: seconds, m/s: meters per second

Primary outcome measures

Upper-body asymmetry metrics and a set of temporal stride parameters were extracted from each stride segmented signal. Table 1 provides a summary of all parameters utilized and their definitions. For the upper body asymmetry parameters, the stride split vertical displacement trajectories of the head (H), withers (W) and pelvis (P) were used. For all three locations, the difference between the vertical displacement minima (HDmin, WDmin and PDmin) and vertical displacement maxima (HDmax, WDmax and PDmax) were calculated (see figure 2 for the walk and tölt examples).

The time-normalized stride data were used to calculate the temporal stride parameters, with the exception of stride speed and stride duration. Footfalls of the individual limbs (left front (LF), right front (RF), left hind (LH) and right hind (RH)) are displayed as %StrD, where LH impact is always at 0 %StrD. The time-normalized stride was also used to calculate the transitions between contralateral limb pairs and the temporal disparities between footfalls within different limb pairs (diagonal and side dissociation).

Statistical analysis

To investigate the impact of lameness induction on both the kinematic and temporal stride parameters listed in table 1, linear mixed models were used for the analysis of both conditions (in-hand, ridden) individually, incorporating all gaits executed during that condition. In addition, linear mixed models were created for the ridden condition where the tölt strides were split into TT, TLC and TMix. Stride-level data for all variables were entered into the model from the baseline and induced lameness measurements. The models were calculated in R-studio (version 1.1.414, RStudio Inc, Boston, Massachusetts, USA) using package nlme (version 3.1-152). In each model, the factor 'lameness' (baseline or induction) was used as the fixed effect and 'gait' (walk, trot or tölt) as interaction. Furthermore, 'horse' was used as a random intercept. To correct for speed differences between baseline and induction and to improve model fit, 'stride speed' was used as the random slope. Correlations between different gaits for the same horse were estimated using an autocorrelation component in each model. Model fit was evaluated using AIC values, q-q plots and residual plotting. Model estimates were represented as estimated marginal means (EMmeans), statistical significance was set at p-value < 0.05 and p-values were adjusted for multiple comparisons using the false discovery rate procedure[20]. Results were displayed in EMmeans or percentage change.

The sensitivity and specificity of the variables mentioned in table 1 were calculated using package pROC (version 1.18) in order to find kinematic and temporal parameters that, on a group level, would probably be linked with lameness at the different gaits. The 10 median strides for each horse were chosen under the baseline and induced lameness circumstances. The difference between the baseline and induction conditions was then used to calculate the sensitivity and specificity for each variable. Using Youden's index, the ideal sensitivity and specificity were computed, along with the matching threshold.

Results

A total of eleven horses (7 geldings, 4 mares, age 5 – 26 years, height at the withers 142 ± 2.5 cm) were used for the analyses of the effects of lameness on temporal and kinematic gait parameters. The average speed for each condition is presented in Table 2. Some data needed to be excluded, an overview of the missing trials is presented in supplementary table 1.

		Trial	
Gait	**Condition**	**Baseline**	**Induction**
Walk	In-hand	1.63	1.68
	Ridden	1.53	1.54
Trot	In-hand	3.68	3.75
	Ridden	4.07	4.15
Tölt	Ridden	3.55	3.58

Table 2. The average speed (m/s) for each condition.

Table 3. in hand walk and trot statistical results. Results are displayed as Estimated marginal means [lower border confidence interval - upper border confidence interval]. * p < 0.01

Kinematic parameters

All effects of induced lameness on kinematic parameters for each gait can be found in table 3 (in-hand trials) and 4 (ridden trials).

Upper-body vertical movement asymmetry results of the in-hand and ridden trials are shown in figure 3. For all gaits, except walk in-hand, lameness induction significantly increased PDmin at walk ($\text{PDmin}_{\text{ridden}}$-0.55 to -4.52 mm), trot ($\text{PDmin}_{\text{hand}}$ -3.9 to -14.7mm, $\text{PDmin}_{\text{ridden}}$-0.67 to –10.52 mm) and tölt, ($\text{PDmin}_{\text{ridden}}$--0.15 to –4.73 mm). However, PDmax only increased significantly in

		Walk			Trot		
VARIABLE	**Units**	**Baseline**	**Induction**	**Difference**	**Baseline**	**Induction**	**Difference**
Kinematic							
HDmin	mm	-3.22 [-8.32 - 1.89]	-6.31 [-11.33 - -1.3]	-3.10*	-3.68 [-7.9 - 0.54]	-1.41 [-5.63 - 2.81]	2.27
WDmin	mm	1.23 [-0.88 - 3.33]	3 [0.91 - 5.08]	1.77*	3.58 [0.24 - 6.92]	7.71 [4.37 - 11.05]	4.13*
PDmin	mm	-0.77 [-5.81 - 4.27]	-1.24 [-6.31 - 3.83]	-0.47	-3.94 [-7.71 - -0.17]	-14.67 [-18.46 - -10.87]	-10.72*
HDmax	mm	1.53 [-2.32 - 5.38]	-2.53 [-6.29 - 1.23]	-4.06*	-2 [-6.22 - 2.22]	-5.67 [-9.87 - -1.48]	-3.67*
WDmax	mm	-1.4 [-3.61 - 0.8]	0.91 [-1.29 - 3.11]	2.32*	-0.48 [-2.53 - 1.58]	-1.53 [-3.57 - 0.5]	-1.06
PDmax	mm	-2.63 [-5.49 - 0.23]	-0.21 [-3.03 - 2.6]	2.41*	-3.36 [-7.5 - 0.78]	-8.16 [-12.26 - -4.07]	-4.80*
hiphike stance	mm				-3.44 [-11.11 - 4.22]	-25.05 [-32.56 - -17.55]	-21.61*
hiphike swing	mm				-5.47 [-14.41 - 3.47]	-26.36 [-35.28 - -17.45]	-20.90*
Head ROMz	mm	74.94 [67.28 - 82.6]	73.38 [65.67 - 81.09]	-1.56	49.22 [44.18 - 54.26]	43.95 [38.87 - 49.02]	-5.27*
Withers ROMz	mm	43.17 [38.45 - 47.9]	46.12 [41.36 - 50.89]	2.95*	51.09 [47.14 - 55.04]	50.19 [46.23 - 54.15]	-0.90
Pelvis ROMz	mm	62.05 [56.12 - 67.99]	68.42 [62.44 - 74.39]	6.36*	48.99 [43.61 - 54.37]	56.78 [51.42 - 62.14]	7.79*
Temporal							
Stance duration LF	S	0.53 [0.5 - 0.55]	0.52 [0.49 - 0.55]	-0.01	0.23 [0.22 - 0.25]	0.24 [0.23 - 0.25]	0.00
Stance duration RF	S	0.51 [0.43 - 0.58]	0.53 [0.46 - 0.61]	0.02	0.2 [0.17 - 0.22]	0.21 [0.18 - 0.23]	0.01*
Stance duration LH	S	0.55 [0.53 - 0.57]	0.54 [0.53 - 0.56]	-0.01	0.24 [0.24 - 0.25]	0.25 [0.24 - 0.25]	0.00
Stance duration RH	S	0.56 [0.54 - 0.58]	0.56 [0.55 - 0.58]	0.01	0.25 [0.24 - 0.26]	0.25 [0.24 - 0.26]	0.00
RH on	%StrD	49.49 [49.01 - 49.97]	49.43 [48.95 - 49.91]	-0.06	49.75 [48.98 - 50.52]	49.27 [48.5 - 50.04]	-0.48*
LF on	%StrD	22.59 [21.06 - 24.13]	22.59 [21.06 - 24.12]	0.00	46.35 [44.77 - 47.94]	46.44 [44.85 - 48.02]	0.09
RF on	%StrD	-27.46 [-29 - -25.93]	-27.54 [-29.07 - -26.01]	-0.08	-4.13 [-5.69 - -2.57]	-4.62 [-6.17 - -3.06]	-0.49*
Transition RF to LF	%StrD	-13.12 [-13.94 - -12.29]	-13.23 [-14.05 - -12.4]	-0.11	3.24 [1.7 - 4.78]	3.89 [2.38 - 5.39]	0.65*
Transition LF to RF	%StrD	-13.8 [-14.66 - -12.95]	-13.57 [-14.43 - -12.72]	0.23	1.75 [0 - 3.5]	0.98 [-0.73 - 2.7]	-0.77*
Transition RH to LH	%StrD	-10.69 [-11.51 - -9.87]	-10.91 [-11.72 - -10.09]	-0.22	4.51 [3.48 - 5.54]	5.58 [4.55 - 6.61]	1.07*
Transition LH to RH	%StrD	-11.93 [-12.72 - -11.14]	-12.1 [-12.89 - -11.32]	-0.17	3.96 [2.87 - 5.05]	3.76 [2.67 - 4.86]	-0.20
Sound diagonal dissociation	%StrD	-26.83 [-25.33 - -28.33]	-26.49 [-24.99 - -27.98]	0.34	-3.5 [-1.84 - -5.15]	-2.95 [-1.3 - -4.6]	0.55*
Lame diagonal dissociation	%StrD	-27.62 [-26.08 - -29.15]	-27.68 [-26.15 - -29.21]	-0.06	-3.8 [-2.26 - -5.34]	-4.27 [-2.73 - -5.8]	-0.47
Sound side dissociation	%StrD	22.87 [21.37 - 24.38]	22.82 [21.32 - 24.33]	-0.05	46.72 [45.16 - 48.28]	46.92 [45.37 - 48.47]	0.20
Lame side dissociation	%StrD	22.47 [20.95 - 23.99]	22.66 [21.14 - 24.18]	0.19	46.31 [44.72 - 47.9]	46.32 [44.74 - 47.91]	0.01

trot in-hand (PDmax$_{\text{hand}}$-3.36 to –8.16mm). Hip-hike also had a significant increase at trot (hip hike swing $_{\text{hand}}$ -5.47 to –26.36 mm, hip hike stance$_{\text{hand}}$ -3.44 to –25.05 mm, hip hike swing$_{\text{ridden}}$ –0.49 to –10.67 mm, hip hike stance$_{\text{ridden}}$ 1.79 to –8.16 mm) and tölt (hip hike swing$_{\text{ridden}}$ –1.80 to –6.08 mm, hip hike stance$_{\text{ridden}}$ –5.42 to –11.03 mm).

HDmin only increased significantly during in-hand walk (-3.22 to -6.31 mm). HDmax increased at walk (walk$_{\text{in-hand}}$ 1.53 to -2.53 mm) and trot (trot$_{\text{in-hand}}$ -2.00 to -5.67 mm, trot$_{\text{ridden}}$ 6.42 to -7.71 mm). WDmin increased at walk (walk$_{\text{in-hand}}$ 1.23 to 3.00, walk$_{\text{ridden}}$ -0.43 to 1.41 mm) and trot (trot$_{\text{in-hand}}$ 3.58 to 7.71 mm, trot$_{\text{ridden}}$ 0.45 to 6.03 mm) but not at tölt. WDmax decreased in walk in-hand (-1.40 to 0.91 mm) and increased only for tölt (4.32 to 5.91 mm).

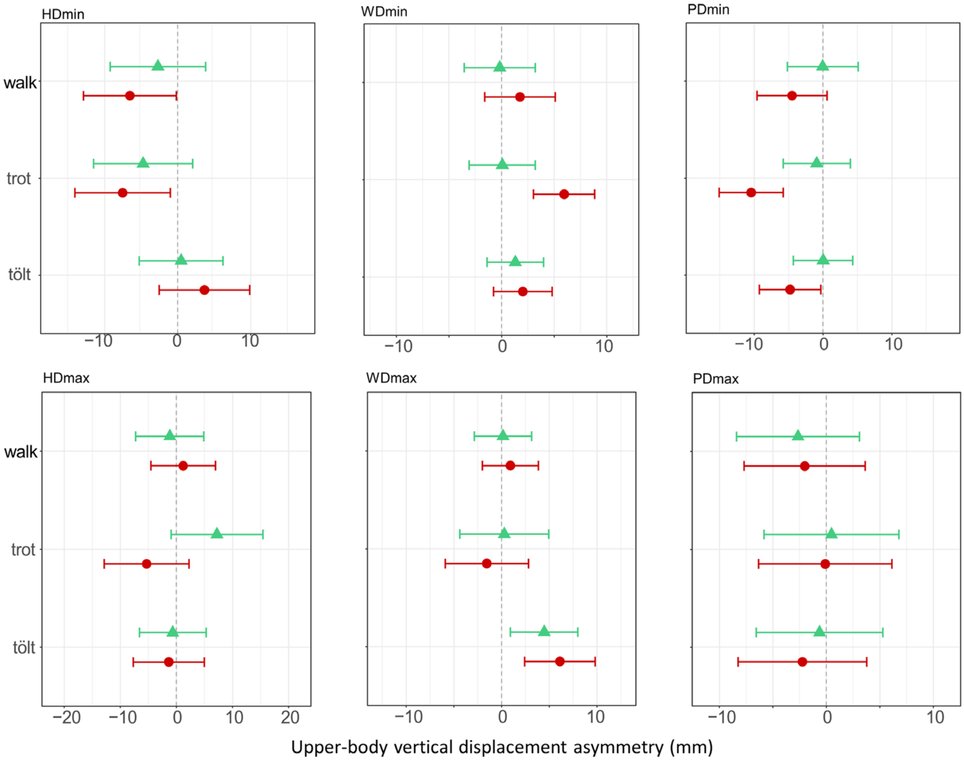

Figure 3. Linear mixed model results (Estimated marginal means and confidence intervals) of upper-body asymmetry values at ridden walk, trot and tölt, before (red circle) and after (blue triangle) hindlimb lameness induction. Upper body asymmetry values of the head (H), withers (W) and pelvis (P) are given in millimeters (mm). Both the difference between the two vertical displacement minima (HDmin, WDmin and PDmin) and between the vertical displacement maxima (HDmax, WDmax and PDmax) are shown. The data represent group (n = 11) estimated marginal means (EMmeans) with the 95% confidence interval as cross hairs.

Table 4. Ridden walk and trot statistical results.Note: Results are displayed as estimated marginal means [lower border confidence interval to upper border confidence interval]. The data represent group(n 10) estimated marginal means (EMmeans) with the = 95% confidence interval [lower border to upper border]. Abbreviations: LF, left forelimb; LH, left hindlimb; RF, right forelimb; RH, right hindlimb; ROMz, vertical range of motion; %StrD, percentage of the stride duration (calculated from LH-on to LH-on moments). *p < 0.01

VARIABLE	Units	WALK			TROT			TÖLT		
		Baseline	Induction	Difference	Baseline	Induction	Difference	Baseline	Induction	Difference
Kinematic										
HDmin	mm	-2.94 [-7.08 - 1.2]	-5.84 [-9.99 - -1.69]	-2.90	-4.08 [-8.27 - 0.1]	-6.62 [-10.66 - -2.57]	-2.53	0.82 [-2.71 - 4.34]	3.5 [-0.36 - 7.36]	2.68
WDmin	mm	-0.43 [-2.52 - 1.66]	1.41 [-0.69 - 3.51]	1.85*	0.45 [-1.47 - 2.37]	6.03 [4.25 - 7.82]	5.58*	1.25 [-0.42 - 2.91]	2.03 [0.29 - 3.77]	0.78
PDmin	mm	0.55 [-2.67 - 3.77]	-4.52 [-7.75 - -1.29]	-5.07*	-0.67 [-3.7 - 2.35]	-10.52 [-13.42 - -7.63]	-9.85*	0.15 [-2.55 - 2.85]	-4.73 [-7.55 - -1.92]	-4.88*
HDmax	mm	-1.56 [-5.46 - 2.35]	0.93 [-2.93 - 4.79]	2.49	6.42 [1.24 - 11.59]	-7.71 [-12.47 - -2.94]	-14.12*	-0.58 [-4.26 - 3.1]	-2.14 [-6.11 - 1.84]	-1.56
WDmax	mm	0.59 [-1.33 - 2.52]	1.29 [-0.61 - 3.2]	0.70	0.46 [-2.58 - 3.5]	-1.99 [-4.87 - 0.89]	-2.45	4.32 [2.03 - 6.61]	5.91 [3.5 - 8.33]	1.59*
PDmax	mm	-3.11 [-6.98 - 0.76]	-2.21 [-6.06 - 1.64]	0.90	-0.48 [-4.63 - 3.66]	-0.55 [-4.66 - 3.56]	-0.07	-0.9 [-4.8 - 3.01]	-2.24 [-6.22 - 1.74]	-1.35
hiphike stance	mm				1.79 [-3.22 - 6.8]	-8.16 [-12.88 - -3.45]	-9.95*	-5.42 [-10.03 - -0.81]	-11.03 [-15.83 - -6.24]	-5.61*
hiphike swing	mm				-0.49 [-5.75 - 4.77]	-10.67 [-15.51 - -5.82]	-10.18*	-1.8 [-6.29 - 2.69]	-6.08 [-10.74 - -1.43]	-4.28*
Head ROMz	mm	66.33 [58.78 - 73.88]	66.25 [58.78 - 73.72]	-0.07	69.43 [60.85 - 78]	66.5 [57.98 - 75.03]	-2.92	69.01 [61.18 - 76.85]	65.06 [57.1 - 73.02]	-3.95*
Withers ROMz	mm	25.34 [21.76 - 28.91]	23.81 [20.23 - 27.39]	-1.53	31.95 [28.2 - 35.7]	34.8 [31.11 - 38.5]	2.86*	35.83 [32.33 - 39.34]	33.35 [29.79 - 36.9]	-2.49*
Pelvis ROMz	mm	63.45 [59.58 - 67.31]	67.89 [63.87 - 71.92]	4.44*	49.95 [46.9 - 53]	50.68 [47.83 - 53.52]	0.72	35.28 [32.41 - 38.14]	40.31 [37.37 - 43.25]	5.03*
Temporal										
Stance duration LF	S	0.54 [0.52 - 0.56]	0.55 [0.53 - 0.57]	0.01	0.26 [0.24 - 0.27]	0.27 [0.25 - 0.28]	0.01*	0.27 [0.25 - 0.28]	0.27 [0.26 - 0.29]	0.01*
Stance duration RF	S	0.55 [0.53 - 0.57]	0.56 [0.54 - 0.58]	0.01	0.25 [0.24 - 0.27]	0.26 [0.24 - 0.27]	0.01*	0.26 [0.24 - 0.28]	0.27 [0.25 - 0.28]	0.01
Stance duration LH	S	0.55 [0.54 - 0.57]	0.58 [0.56 - 0.6]	0.03*	0.26 [0.25 - 0.26]	0.27 [0.26 - 0.28]	0.01*	0.29 [0.28 - 0.3]	0.29 [0.29 - 0.3]	0.01
Stance duration RH	S	0.55 [0.54 - 0.57]	0.58 [0.56 - 0.6]	0.03*	0.26 [0.25 - 0.28]	0.28 [0.27 - 0.29]	0.01*	0.3 [0.29 - 0.31]	0.3 [0.29 - 0.31]	0.01*
RH on	%StrD	49.29 [48.81 - 49.77]	49.53 [49.06 - 50]	0.24	50.52 [49.87 - 51.18]	49.92 [49.27 - 50.58]	-0.60*	49.64 [49.07 - 50.21]	49.7 [49.1 - 50.3]	0.06
LF on	%StrD	22.27 [20.17 - 24.37]	23.13 [21.03 - 25.23]	0.86*	44.09 [41.32 - 46.86]	47.74 [44.39 - 51.09]	3.65*	18.63 [16.49 - 20.77]	18.17 [16 - 20.33]	-0.47
RF on	%StrD	-28.9 [-30.85 - -26.94]	-28.24 [-30.2 - -26.28]	0.65*	-5.48 [-7.63 - -3.34]	-5.22 [-7.31 - -3.13]	0.26	-32.6 [-34.49 - -30.7]	-33.57 [-35.49 - -31.65]	-0.98*
Transition RF to LF	%StrD	-14.43 [-15.1 - -13.76]	-15.02 [-15.67 - -14.37]	-0.59*	-3.57 [-4.62 - -2.53]	-3.1 [-4.17 - -2.02]	0.48	-4.22 [-5.12 - -3.32]	-5.01 [-5.96 - -4.06]	-0.79*
Transition LF to RF	%StrD	-12.99 [-13.98 - -12.01]	-13.16 [-14.13 - -12.2]	-0.17	-2.68 [-3.85 - -1.51]	-5.1 [-6.29 - -3.91]	-2.42*	-3.56 [-4.6 - -2.51]	-4.94 [-6.02 - -3.86]	-1.39*
Transition RH to LH	%StrD	-11.93 [-12.67 - -11.2]	-12.63 [-13.36 - -11.9]	-0.70*	-3.91 [-4.85 - -2.97]	-4.32 [-5.25 - -3.39]	-0.41	-7.6 [-8.4 - -6.8]	-8.05 [-8.88 - -7.22]	-0.45*
Transition LH to RH	%StrD	-13.64 [-14.31 - -12.97]	-13.73 [-14.39 - -13.06]	-0.09	-3.07 [-3.97 - -2.17]	-4.25 [-5.15 - -3.36]	-1.18*	-8.89 [-9.66 - -8.13]	-9.11 [-9.91 - -8.3]	-0.21
Sound diagonal dissociation	%StrD	-27.19 [-25.65 - -28.74]	-26.31 [-24.75 - -27.87]	0.89*	-3.81 [-2.15 - -5.46]	-2.06 [-0.49 - -3.62]	1.75*	-31.58 [-30.12 - -33.05]	-32.33 [-30.84 - -33.83]	-0.75
Lame diagonal dissociation	%StrD	-28.12 [-25.9 - -30.34]	-27.48 [-25.26 - -29.7]	0.64	-6.4 [-3.78 - -9.02]	-3.76 [-1.14 - -6.39]	2.64*	-31.81 [-29.56 - -34.05]	-32.64 [-30.38 - -34.9]	-0.84*
Sound side dissociation	%StrD	21.79 [20.05 - 23.53]	22.33 [20.59 - 24.08]	0.55	42.23 [39.88 - 44.57]	44.69 [42.6 - 46.78]	2.46*	17.85 [16.16 - 19.55]	17.18 [15.46 - 18.89]	-0.68*
Lame side dissociation	%StrD	21.91 [19.97 - 23.86]	22.89 [20.94 - 24.84]	0.97*	43.98 [41.33 - 46.64]	47.78 [44.71 - 50.85]	3.80*	18.33 [16.37 - 20.29]	17.92 [15.94 - 19.91]	-0.40

Vertical range of motion (ROMz; mm) of the head did not change at walk but decreased at trot in-hand (49.22 to 43.95 mm) and tölt (69.01 to 65.06 mm). Withers ROMz increased at walk in-hand (walk$_{\text{in-hand}}$ 43.17 to 46.12 mm) and increased at ridden trot (trot$_{\text{ridden}}$31.95 to 34.80 mm), but decreased in tölt (35.83 to 33.35 mm). Pelvis ROMz increased at walk (walk$_{\text{in-hand}}$ 62.05 to 68.42 mm, walk$_{\text{ridden}}$ 63.45 to 67.89 mm) in-hand trot (trot$_{\text{in-hand}}$ 48.99 to 56.78 mm) and tölt (35.28 to 40.31 mm).

Temporal parameters

The effects of induced lameness on temporal stride parameters for each gait are summarized in figure 4, and statistical results can be found in table 3 for in-hand conditions and table 4 for ridden conditions.

At in-hand walk, no changes in limb timing variables were found. At ridden walk, the stance duration of both hindlimbs increased (0.55 to 0.58 s), the transition time from RF to LF increased, and the transition time from LH to RH increased. Also, both the lame and sound side dissociation changed from 21.91 to 22.89 %StD and from -27.19 to -26.31 %StD, respectively.

At trot in-hand, the stance duration increased for RF (0.20 to 0.21 s) and transition time from LF to RF decreased (1.75 to 0.98 %StD), from RF to LF increased (3.24 to 3.89 %StD) and from RH to LH increased (4.51 to 5.58 %StD). At ridden trot, stance duration increased for all limbs: LF (0.26 to 0.27 s), RF (0.25 to 0.26 s), LH (0.26 to 0.27 s) and RH (0.26 to 0.28 s). The transition time from LF to RF decreased (-2.68 to -5.1 %StD) and the transition time from LH to RH decreased (-3.07 to -4.25 %StD). Negative values indicate that there is a double forelimb and hindlimb support. The sound diagonal dissociation decreased from -3.81 to -2.06 %StD and lame diagonal dissociation from -6.40 to -3.76 %StD respectively. Here, negative values indicate that the forelimb lands first. In addition, sound and lame side dissociation increased (42.23 to 44.69 %StD, 43.98 to 47.78 %StD respectively).

At tölt, stance duration increased significantly for LF (0.27 to 0.27 s) and RH (0.3 to 0.3 s). Transition time from RF to LF (-4.22 to-5.01 %StD) and LF to RF (-3.56 to -4.94 %StD) increased where negative values indicate a double forelimb support. Transition time for RH to LH (-7.6 to -8.05 %StD) also increased. Lame diagonal dissociation increased (-31.81 to -32.64 %StD) and sound side dissociation decreased (17.85 to 17.17 %StD).

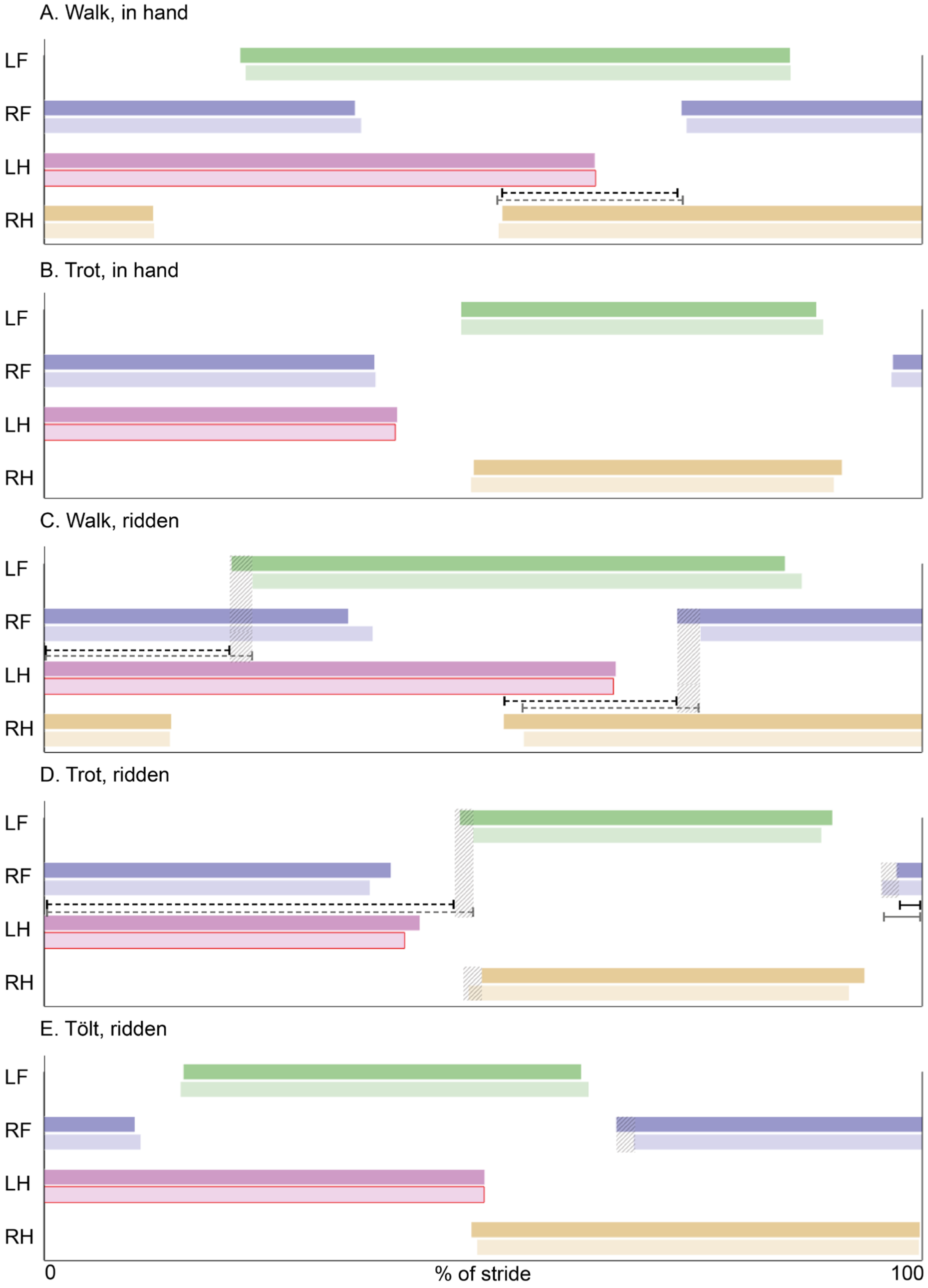

Figure 4. Graphical representation of the temporal changes. Footfall patterns during (A) walk in-hand, (B) trot in-hand, (C) ridden walk, (D) ridden trot and (E) ridden tolt, before (filled) and after (shaded) lameness induction of the left hind (LH) limb. Significant changes in the footfall of the right front (RF) limb are displayed with the grey hatched area, which relates to the significant changes in diagonal dissociation (DD) (solid lines) and side dissociation (SD) (dashed lines). Black lines represent the baseline DD and SD, whereas grey lines represent DD and SD after induction. The data represents the group (n = 10 for in-hand trials and n = 9 for ridden trials) mean footfall patterns.

Results of split tölt

The effects of induced hindlimb lameness on types of tölt (TT, TLC and TMix) are summarized in supplementary table S2.

Sensitivity and specificity

The sensitivity and specificity results can be found in supplementary table S3 and S4 and were also plotted in ROC curves (Fig S2-4). For walk in-hand, PDmin was a reliable kinematic variable (specificity = 0.62, sensitivity = 0.75) and lame diagonal dissociation (specificity = 0.61, sensitivity = 0.65) was a good temporal variable to discriminate between baseline and induction. At ridden walk, PDmin was the most sensitive and specific (specificity = 1.0, sensitivity = 0.88) kinematic variable and sound side dissociation was a good temporal variable (specificity = 0.87, sensitivity = 0.65). For trot in-hand, Hip Hike stance (specificity = 0.84, sensitivity = 0.93) was the best variable and Hip Hike swing (specificity = 0.86, sensitivity = 0.80) and PDmin (specificity = 0.84, sensitivity = 0.92) were reliable variables too. For ridden trot, Hip Hike stance (specificity = 0.80, sensitivity = 0.80) WDmin (specificity = 0.84, sensitivity = 0.931) and PDmin were reliable variables (specificity = 1.0, sensitivity = 0.91). For tölt, kinematic variables had either low specificity, low sensitivity or both. For temporal variables, the transition time from LF to RF seems to be most sensitive for tölt (specificity = 0.58, sensitivity = 0.78).

Discussion

The study examined the effect of induced hindlimb lameness on Icelandic horses' limb kinematics and upper-body movement during various gaits. We hypothesized that Icelandic horses would exhibit upper-body vertical asymmetries in response to hindlimb lameness comparable to those of hindlimb lame horses at trot. This is the first study that describes the effect of induced hindlimb lameness on the gait kinematics of Icelandic horses during walk, trot and tölt. The horses were led in-hand for walk and trot, which is similar to the lameness examination situation, but also ridden at walk, trot and tölt, before and after lameness induction.

Our results support our hypothesis that Icelandic horses show compensatory mechanisms to induced hindlimb lameness at all gaits, where during trot in-hand the Icelandic horses showed similar increases in vertical pelvic movement asymmetry as shown in earlier studies of other breeds[12,21]. In the present study, PDmin showed the largest increase for both in-hand trot and the three ridden conditions, reflecting mainly an impact lameness induced by the sole-pressure model. PDmax only increased slightly for in-hand trot and not for the other conditions. A compensatory ipsilateral HDmax asymmetry was seen during both in-hand and ridden trot in accordance with Rhodin et al. (2013)[12]. Though in contrast, no change in HDmin was seen in the Icelandic horses. In a large study of 317 lame horses, including 10 Icelandic horses, a compensatory HDmin, but no compensatory HDmax asymmetry was seen in the horses that were present with either a PDmin (n=59) and/or PDmax (n=85) as a result of lameness confirmed with a positive block.[22] The reason for the elevated head position after the lame diagonal push-off is unclear, though we hypothesize that this helps to pitch the trunk backwards to shift the center of mass caudally (within diagonal compensation). Another explanation could be that the forelimbs are generating higher forces to push the body upwards and forward. This may explain why the compensatory diagonal WDmax asymmetry seen in the study by Persson-Sjodin et al.[13] was not found for the Icelandic horses in the present study at trot. The HDmax compensatory strategy seen in Icelandic horses seems to be different compared to other horse breeds, but this needs further investigation in clinically lame Icelandic horses. Head ROMz decreased, despite an increase in HDmax, and pelvic ROMz increased after lameness induction during in-hand trot as seen in earlier studies[4,23]. Though during ridden trot, only withers ROMz increased slightly. The hindlimb lameness induction significantly increased the hip hike at in-hand trot, both during the stance and the swing phase, where the magnitude of the change was almost twice as large compared to the changes in PDmin. Therefore, we suggest that the hip hike may be more useful to visually assess Icelandic horses during a lameness exam when trotting in-hand.

This study also described the adaptation strategies to hindlimb lameness at walk. More kinematic changes after hindlimb lameness induction were found at ridden walk compared to in-hand walk. Overall, the changes after induction were very small, probably too small to be visible to the naked eye. The reason could be that the lameness induced was very subtle, but increased with the excessive weight of the rider, increasing the magnitude of the asymmetries during ridden walk. During in-hand walk, pelvis vertical movement only slightly changed. As such, pelvic ROMz increased, no changes were seen in PDmin, and only a very small change in direction of the PDmax was found, though this remained close to zero. Compensatory ipsilateral HDmin and HDmax increases were observed, and WDmin increased slightly during in-hand walk, with a lower withers position during double forelimb support when the right forelimb was retracted. WDmax changed direction to positive, though remained very close to symmetric. During ridden walk, PDmin asymmetry increased after induction. The pelvis reached a lower position during double hind limb support with the lame hindlimb retracted. This can be explained using walking mechanics, as peak hindlimb loading takes place at the beginning of the stance phase at walk[24]. For in-hand walk no temporal stride variables changed. Whereas for ridden walk, stance duration for both hindlimbs increased. Sound diagonal dissociation decreased and lame side dissociation decreased.

At the tölt, the changes in kinematics were small and of similar magnitude as those found at walk. A possible explanation is that the experienced pain could be lower, as during tölt, lower peak vertical forces are observed compared to trot[25]. Another explanation could be that Icelandic horses have more difficulties in compensating for hindlimb lameness at tölt, due to the unilateral support phases of each limb. Still, some adaptations were observed. A small increase in PDmin asymmetry was found and the hip hike increased both during swing and stance in a similar way as during trot, but of a smaller magnitude. The head and withers ROMz decreased at tölt, while pelvis ROMz increased after lameness induction, similar to what was found for walk and trot in-hand. The stance duration increased for the left forelimb and the (non-induced) right hindlimb. Transition time from RF to LF, LF to RF and RH to LH increased resulting in longer periods of bilateral forelimb and bilateral hindlimb support.

Conclusion

PDmin and hip hike have proven to be effective parameters for assessing hindlimb lameness in Icelandic horses during both in-hand and ridden trot. However, during ridden walk and tölt, PDmin exhibited only minor changes. These variations could be attributed to differences in single or bipedal hindlimb support, as pelvic minimum position indicates different distinct loading stages throughout the stride cycle across various gaits.

These findings hold great importance for clinicians evaluating lameness in real-world settings, whether they are using advanced gait analysis technologies or not. Our research suggests that when assessing lameness in Icelandic horses, it is best to do so at a trot rather than tölt, whenever feasible. If horses resist trotting when led by a handler, having a rider perform a sitting trot can aid in the collection of valuable kinematic data for assessment of upper-body symmetry parameters such as PDmin and hip hike.

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Ethics: The experimental procedure and data collection were approved by the ethical committee of the Icelandic Food and Veterinary Authorities, (no 2018-02-01). Informed written consent was obtained from the owner of the animals and no data from human participants were included in this study.

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5

Continuous versus discrete data analysis for gait evaluation of horses with induced bilateral hindlimb lameness

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Abstract

Background: Gait kinematics measured during equine gait analysis are typically evaluated by analysing (asymmetry-based) discrete variables (e.g., peak values) obtained from continuous kinematic signals (e.g., timeseries of datapoints). However, when used for the assessment of complex cases of lameness, such as bilateral lameness, discrete variable analysis might overlook relevant functional adaptations.

Objectives: The overall aim of this paper is to compare continuous and discrete data analysis techniques to evaluate kinematic gait adaptations to lameness.

Study design: Method comparison.

Methods: Sixteen healthy Shetland ponies, enrolled in a research programme in which osteochondral defects were created on the medial trochlear ridges of both femurs, were used in this study. Kinematic data were collected at trot on a treadmill before and at 3 and 6 months after surgical intervention. Statistical parametric mapping and linear mixed models were used to compare kinematic variables between and within timepoints.

Results: Both continuous and discrete data analyses identified changes in pelvis and forelimb kinematics. Discrete data analyses showed significant changes in hindlimb and back kinematics, where such differences were not found to be significant by continuous data analysis. In contrast, continuous data analysis provided additional information on the timing and duration of the differences found.

Main limitations: A limited number of ponies were included.

Conclusions: The use of continuous data provides additional information regarding gait adaptations to bilateral lameness that is complementary to the analysis of discrete variables. The main advantage lies in the additional information regarding time dependence and duration of adaptations, which offers the opportunity to identify functional adaptations during all phases of the stride cycle, not just the events related to peak values.

Keywords: clinical; data analysis; gait analysis; horse; kinematics

Introduction

Currently, quantitative gait analysis systems for clinical lameness evaluations in horses rely on the detection of movement asymmetries between left and right.[1] Typically, 3-dimensional (3D) kinematic signals are recorded, separated into multiple continuous 2D angle-time or displacement-time signals and then further analysed by extracting single (peak) values. Using this approach, the horses' complex motion pattern is reduced to a manageable amount of scalar, time discrete variables.

Several kinematic and kinetic differences between the locomotion of healthy and unilaterally lame horses have already been identified.[1] These include decreased vertical displacement of the head, withers and/or pelvis during the stance phase of the lame limb,[2-4] increased upward movement of the tuber coxae before touchdown of the affected limb (hip hike)[5] and reduced peak vertical force (PVF) of the affected limb,[6,7] all resulting in movement asymmetry. However, these discrete variables represent only a small part of the horse's movement and when an asymmetric pattern is absent, such as in cases of bilateral lameness,[8] analyses based solely on such discrete variables may be insufficient to discriminate between healthy and lame horses.

The reliance on discrete variables to identify gait adaptations to lameness has three limitations. Firstly, adaptations may occur over phases of the stride that cannot be described by single discrete variables. Secondly, the timing of single values can differ between sides without changing in amplitude. And thirdly, discrete variables are not necessarily independent and analysing them as such may result in bias.[9] To overcome these limitations, continuous data analysis techniques,[10] such as statistical parametric mapping (SPM),[9] have been developed. To assess the value of continuous data analysis for identifying functional adaptations to lameness in general and more specifically to bilateral hindlimb lameness in equine locomotion analysis, a comparison of kinematic findings retrieved from continuous versus discrete analyses is warranted.

The purpose of the current study was to compare results from continuous and discrete data analysis techniques to evaluate kinematic adaptations to induced bilateral hindlimb lameness. We hypothesised that continuous data analysis techniques would provide more detailed information about functional kinematic adaptations compared to the analysis of discrete values.

Materials and methods

Animals

Sixteen sound Shetland ponies were used in this study. All ponies were enrolled in an articular cartilage repair study in which they underwent a surgical intervention to create osteochondral defect bilaterally on the medial trochlear ridges of both femurs and treated by the implantation of a bio-engineered scaffold.[11] All were mares, with an age distribution of 4-12 years and a mean ± SD body mass of 169 ± 29 kg.

Data collection

Prior to the experiment, the ponies were accustomed to treadmill exercise.[12] Kinematic data were recorded using six infra-red 3D optical motion capture (OMC) cameras (Qualisys AB, Motion Capture Systems) that registered the positions of 28 skin mounted spherical reflective markers (19-24 mm) at 200 Hz. For detailed marker placement, see Figure S1. Data collection lasted 30 s for each trial at trot on a treadmill after a warm-up period at walk and trot. Measurements were performed at the individually preferred trotting speed for each pony, based on visual assessment of locomotion regularity.[12] Subsequent measurements were speed matched, ensuring control over speed along all timepoints. The ponies were measured at three timepoints: prior to the surgical intervention at baseline (T0), and at 3 months (T1) and 6 months (T2) after surgical intervention.

Name	**Units**	**Description**	**Anatomical landmarks**
Body			
MinDiff	mm	Absolute difference between the left and right stride half-cycle in minimum vertical position	Head: poll Withers: T8 Sacrum: Tuber sacrale
MaxDiff	mm	Absolute difference between the left and right stride half-cycle in maximum vertical position	Head: poll Withers: T8 Sacrum: Tuber sacrale
Pelvis roll	deg	Rotation of the pelvis around the longitudinal axis of the horse	Tuber sacrale, left/right tuber coxae
Pelvis pitch	deg	Rotation of the pelvis around the transversal axis	Tuber sacrale, left/right tuber coxae
Pelvis yaw	deg	Rotation of the pelvis around the vertical axis	Tuber sacrale, left/right tuber coxae
Back flexion-extension	deg	Rotation of the back around the transverse axis, with T15 as the point of rotation.	T8, T15, tuber sacrale
Back lateral bending	deg	Rotation of the back around the vertical axis, with T15 as the point of rotation.	T8, T15, tuber sacrale
Limbs			
Protraction	deg	Sagittal movement of the whole limb	Elbow, hoof
Fetlock extension	deg	Sagittal rotation around the fetlock joint	Proximal and distal end of metacarpal/metatarsal bone, hoof

Table 1. Overview of the kinematic variables used. Units are given in millimetres (mm) for displacement variables and degrees (deg) for variables expressed in angles

Data processing

The reconstruction of the 3D coordinates of each marker was automatically calculated by using motion capture software (QTM[a], version 2.9). Each marker was identified and labelled using an automated model and manually checked. Raw data consisting of the 3D data of the designated markers were exported to Matlab (version 2019b) (The MathWorks Inc, Natick, Massachusetts, USA) for further analysis using custom written scripts. Stride segmentation was performed based on the maximal vertical position of the tuber sacrale before maximal protraction of the left hindlimb. All signals were high-pass filtered using a fourth-order Butterworth filter with the cut-off frequency adjusted based on the stride frequency of the individual ponies.[13] Strides with excessive head movement (two SD's from the trial mean) were automatically removed. For further analysis, the first 20 strides of each trial were selected for each pony. Bone segments were formed based on marker locations and angles between these segments were calculated for each stride. See Table 1 for variable definitions. The data were exported as discrete variables (ie, minima, maxima, and range of motion (ROM)) for discrete data analysis and exported as a timeseries of 101 datapoints per stride for continuous data analysis.

Data analysis

For the analysis of discrete variables, stride-level data were analysed in Open software R (version 3.3.1) (R-studio, Boston, Massachusetts, USA), using package lme4 (version 1.1-15) for mixed modelling. In each linear mixed model (LMM), random effect was "pony" and "timepoint" was used as the fixed effect. The dependent variables were investigated for a transformation close to normality using probability plotting and examining for skewness and kurtosis. When non-normally distributed variables were found, these variables were transformed using the Box-Cox method. The model estimates were represented as least squares means and confidence intervals.

For SPM analysis of the kinematic data, the mean value of the curves was subtracted for each timepoint to compensate for possible marker placement errors between trials. The normalised stride values were assembled into 20*101*1 vector fields (20 strides, 101 data points, 1 dimension per data point) for each joint, timepoint, and pony. The open source spm1d package (version M.0.4.1, Pataky, 2012) was used to conduct the SPM analysis in Matlab. Repeated measures ANOVAs were performed to compare kinematics between the three timepoints. If there were significant results, post hoc paired *t* tests were done to determine which timepoints were different. For both the SPM and discrete value analyses, significance was set at *P* value < .05, and *P* values were adjusted for multiple comparisons using the Benjamin-Hochberg procedure.[14]

			T0		T1		T1-T0			T2		T2-T0		
Variable	**Units**	**Location**	**Estimate**	**SE**	**Estimate**	**SE**	**Difference**	**% Difference**	**p-value**	**Estimate**	**SE**	**Difference**	**% Difference**	**p-value**
MinDiff	mm	Head	6.7	0.7	6.8	0.7	0.1		>0.9	6.8	0.7	0.1		>0.9
		Withers	3.9	0.5	4.0	0.5	0.1		>0.9	3.6	0.5	-0.3		0.6
		Pelvis	4.2	0.4	4.7	0.4	0.5		0.2	4.5	0.4	0.4		0.5
MaxDiff	mm	Head	7.9	1.1	7.5	1.1	-0.3		>0.9	7.9	1.1	0.0		>0.9
		Withers	2.7	0.4	3.4	0.4	0.7		<0.001	2.8	0.4	0.1		>0.9
		Pelvis	5.4	1.1	5.4	1.1	0.0		>0.9	5.0	1.1	-0.4		0.5
Vertical ROM	mm	Head	39.3	1.5	38.8	1.5	0.4	-1.1	>0.9	39.8	1.5	-0.5	1.3	0.8
		Withers	34.4	1.4	35.2	1.4	0.9	2.5	0.07	36.7	1.4	2.3	6.8	<0.001
		Pelvis	39.5	1.7	39.8	1.7	0.3	0.8	0.8	39.4	1.7	-0.1	-0.2	>0.9
Flexion - extension	deg	Back	4.6	0.2	4.1	0.2	-0.5	-10.5	<0.001	4.1	0.2	-0.4	-9.5	<0.001
Lateral bending		Back	6.5	0.4	7.1	0.4	0.6	8.8	<0.001	6.9	0.4	0.4	6.3	<0.001
Roll	deg	Pelvis	5.0	0.3	5.4	0.3	0.4	8.2	<0.001	5.6	0.3	0.6	12.6	<0.001
Pitch		Pelvis	7.3	0.4	6.4	0.4	-0.9	-12.2	<0.001	6.0	0.4	-1.4	-18.6	<0.001
Yaw		Pelvis	3.7	0.3	4.1	0.3	0.4	11.6	<0.001	3.9	0.3	0.3	7.4	<0.001
Fetlock extension ROM	deg	LF	-35.0	0.6	-33.4	0.6	1.6	-4.5	<0.001	-32.7	0.6	2.3	-6.6	<0.001
		RF	-35.2	0.6	-33.0	0.6	2.2	-6.3	<0.001	-32.3	0.6	2.9	-8.1	<0.001
		LH	-39.0	1.0	-39.7	1.0	-0.7	1.9	<0.001	-38.7	1.0	0.3	-0.7	0.5
		RH	-38.5	0.9	-37.3	0.9	1.2	-3.0	<0.001	-37.8	0.9	0.7	-1.9	<0.001
Protraction ROM	deg	LF	38.1	0.7	40.0	0.7	2.0	5.2	<0.001	39.4	0.7	1.4	3.6	<0.001
		RF	38.1	0.7	40.0	0.7	1.9	4.9	<0.001	39.3	0.7	1.2	3.0	<0.001
		LH	37.8	0.8	39.5	0.8	-0.2	-0.6	0.4	37.5	0.8	-0.2	-0.7	0.3
		RH	37.3	0.7	28.8	0.7	0.0	-0.1	>0.9	37.4	0.7	0.1	0.3	0.9

Table 2. Linear mixed model (discrete variable analysis) results. LF left forelimb; RF right forelimb; LH left hindlimb; RH Right hindlimb; ROM range of motion. All angle values describe the range of motion. For trunk and hindlimb kinematics: N 14, for = = = = = = forelimb kinematics: N = 13. Units are given in millimetres (mm) for displacement variables and degrees (deg) for variables expressed in angles.

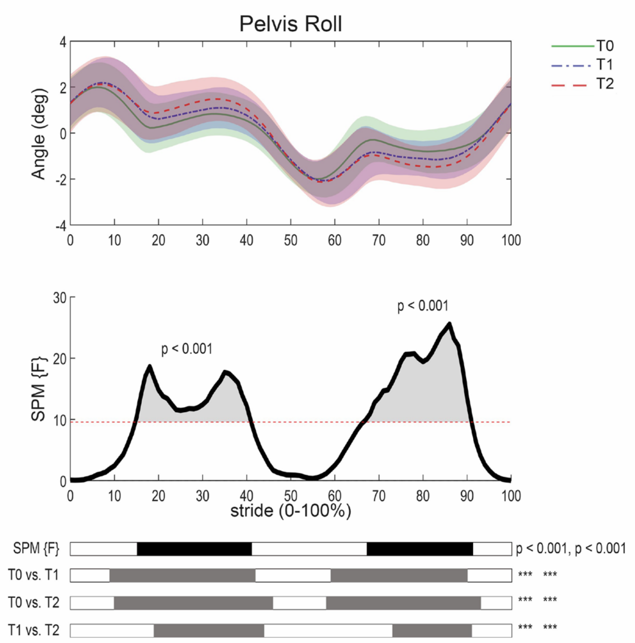
Results

Two ponies were lost from the study: one due to severe lameness, another because no baseline measurement was recorded. Also, due to a misplaced marker, trials for forelimb kinematics were removed for one pony. The mean ± SD trotting speed was 2.18 ± 0.16, 2.21 ± 0.15 and 2.21 ± 0.16 m/s for T0, T1 and T2, respectively. At all timepoints the mean stride duration was 0.54 ± 0.01 s.

Discrete variable analysis

Discrete variable findings for the differences between the three timepoints can be found in Table 2. In terms of symmetry parameters, no significant differences (P > .05) were found between timepoints, except for the maxDiff of the withers at T1 (P < .001), which increased by 0.7 mm. For other kinematic variables, represented as the percentage of change in ROM at T1 and T2 compared to T0, significant differences between timepoints included a decrease in fetlock extension (−4.5% to −8.1%, P < .001) of the forelimbs, back flexion-extension (−9.5% to −10.5%, P < .001) and pelvis pitch (−12.2% to −18.6%, P < .001). Significant increases were found in lateral bending of the back (6.3% to 8.8%, P < .001), pelvis yaw (7.4% to 11.6%, P < .001), pelvis roll (8.2% to 12.6%, P < .001) and protraction of the forelimbs (3.0% to 5.2%, P < .001). The differences in pelvis pitch and forelimb fetlock extension significantly increased over time. In contrast, the differences in pelvis yaw and forelimb protraction peaked at T1 and decreased again at T2 in relation to T0.

Figure 1. Example of summarised presentation of statistical parametric mapping (SPM) results. The upper graph shows the mean kinematic pelvis roll angle at the three timepoints. The middle graph shows the SPM {F} statistic as a function of the gait cycle. The critical threshold (red dashed line) was exceeded between 15%-42% and 67%-91% of the gait cycle. Lower black bars represent a simplified visualisation of the significant areas indicated by the SPM {F} statistic. Grey bars represent a simplified visualisation of the post hoc paired analysis of the differences between all timepoints (ie, post hoc paired t test (SPM {t}), a = 0.0036). *P < .05, **P < .01, ***P < .001



Continuous data analysis

Figure 1 illustrates SPM findings. Representative SPM results are shown in Figure 2, further SPM figures can be found in Figures S2- S5. There were no significant differences between the left and right step (Figures S4 and S5). Between timepoints, significant differences were found in pelvis kinematics (Figure 2A,B), where both at T1 and T2 in relation to T0, pelvis roll (Figure 2A) increased during the stance phase ($P < .001$) and pelvis pitch (Figure 2B) decreased at its extremes during both the stance and swing phase of the right step ($P < .001$). Changes in limb kinematics appeared in the forelimbs (Figure 2C), where the curve was significantly delayed with regard to the stride split at T1 and T2 in relation to T0 ($P < .001$).

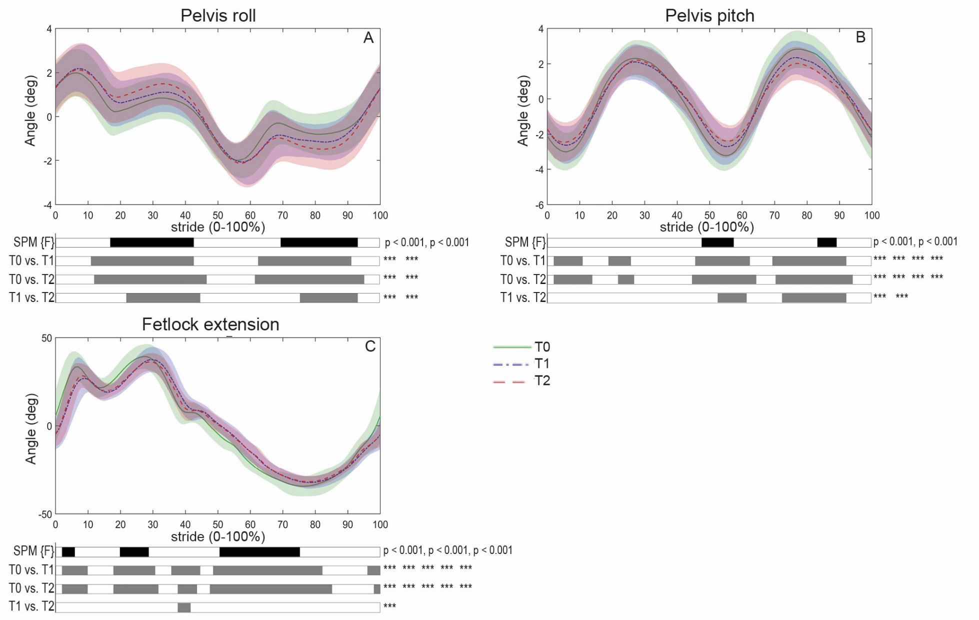


Figure 2. Each graph shows the mean kinematic signal of T0 (green-solid), T1 (blue-dashed) and T2 (red-dashed) at the level of each joint. A, Pelvis roll angle, demonstrating a change outside of peak values. B, Pelvis pitch angle, demonstrating changes on peak values. C, Fetlock extension angles for the right forelimb, demonstrating a time shift of the complete curve. Black bars indicate gait phases during which the SPM {F} statistic exceeded the critical threshold. Grey bars indicate gait phases during which the SPM {t} statistic exceeds the critical threshold in the post hoc analysis (ie, post hoc paired t test, a = 0.0036). $*P < .05$, $**P < .01$, $***P < .001$

Discussion

The purpose of the current study was to compare continuous and discrete data analysis techniques to evaluate gait adaptations in cases of induced bilateral hindlimb lameness. With the use of the discrete variable analysis, more variables were found to change significantly compared to continuous data analysis. However, changes outside the peak value areas and the duration of the changes are not taken into account using discrete variables alone, whereas the continuous data analysis considered both.

Analysis of trunk kinematics indicated there was no change in upper body vertical movement, except for the maxDiff of the withers, which was, however, of minor magnitude that is deemed not clinically relevant.[15] Both types of analysis identified comparable adaptations in pelvis pitch and back flexion-extension kinematics. The change in back flexion-extension was only significant for the discrete variable analysis but a trend was present in the continuous data. The curves for these movements are all sinusoidal and the adaptations are largest at the peaks (Figure 2B). Discrete variable analysis indicated considerable increases in pelvis yaw and back lateral bending, which were not identified using continuous data analysis. For pelvis roll kinematics, both types of analyses indicated an increase in pelvis roll angles. The continuous data analysis showed that time wise the difference was largest from mid- to late stance of each hindlimb (Figure 2A), the discrete analysis only identified the significant difference in ROM. The analysis of continuous data hence provides additional information about the moment and duration of the differences during the stride, which can help us better understand and explain the dynamics of gait adaptations to lameness.

In hindlimb fetlock extension angles, small but significant differences were identified using discrete variable analysis, which were not detected by continuous data analysis. Both methods suggested a decrease in protraction ROM and an increase in fetlock extension ROM for both forelimbs. The SPM results additionally showed the presence of significant delays in the timing of the sagittal plane movement of the forelimbs with regard to stride segmentation (Figure 2C). In this study the stride segmentation is based on maximal vertical displacement of the sacrum, which is tightly related to the timing of hindlimb kinematics.[16] Therefore, it is possible that forelimb kinematics are not delayed, but that the hindlimb placement is advanced, resulting in earlier support of the trunk during the stride cycle, which is consistent with findings of studies on unilateral lameness.[2]

There are several explanations for the differences in findings between continuous and discrete data analysis. Firstly, fewer parameters were found to be significant using SPM compared to LMM. This is concordance with earlier studies comparing discrete to continuous data analysis of under hoof ground reaction forces.[17,18] SPM uses random field theory[19] to ensure a tight control of alpha. This may have resulted in fewer type I errors made compared to LMM,[9] which assumes a point-process Gaussian variance model.[20] Hobbs et al[17] pointed out that, due to this tight control of alpha in SPM analysis, reaching significance may not be as important as understanding the clinical implications of functional adaptations. Secondly, with discrete data analysis, only single points on the extremes of the signal were selected for further analysis. Failure to test differences throughout the complete signal means that relevant adaptations that occurred outside of these extremes might be missed. And lastly, both amplitude and timing of events may have varied between timepoints and individuals. These amplitude and timing variations can be influenced by factors such as skin displacement artefacts,[21] placement of markers[22] and further habituation to the measurement situation.[12] With shifts in timing of events between trials and individuals, the peak angles may be cancelled out when summarising continuous signals. Thus, with variability of movement and fluctuations in timing and amplitude, peak angles become less evident when using continuous data, but not with discrete variables.

The analysis of continuous data may hold additional advantages regarding the evaluation of equine lameness. Firstly, the analysis of continuous signals may help to further differentiate during the lameness exam. This has been shown for equine gait kinetics, where for instance palmar foot syndrome and mild tendinopathy were associated with changes in the shape of the vertical ground reaction force curve, rather than simply a reduction of PVF in the lame limb(s).[23] Hobbs et al[17] also used SPM on the individual kinetic pattern and found subtle asymmetries in the sound horse. They suggest that, if the periods of asymmetry can be related to specific events in the stride cycle, it may help the interpretation of their functional significance. However, subtle asymmetries might be lost when data are averaged over a group of horses, which indicates the importance of evaluating horses on an individual basis.[17] In case of knee osteoarthritis in humans, it has been shown that hip and knee rotation patterns differ between medial and lateral knee osteoarthritis[24] and both joint moment and joint angle patterns change with the severity of the disease.[25] Thus, the timing of the change can be related to the function of the affected structure and may therefore help to localise the pain. Changed joint angle patterns related to decreased joint loading have already been used to alter walking patterns,[26] showing potential for both diagnosis and rehabilitation purposes. Changes of this nature should be detectable using continuous data analysis techniques but are overlooked when only using discrete variables. Secondly, results of kinematic analyses using continuous data may better resemble the daily practice of evaluation of locomotion during lameness exams compared to considering only discrete variables. When assessing individual horses, veterinarians usually follow a structured protocol of observation: systematically assessing the overall movement pattern, then focus on the region of interest (such as the head or pelvis) and afterwards determine at which point in the gait cycle the most obvious abnormality is visible. Further investigations are needed to determine to which extent continuous data analysis techniques agree with clinical assessments of movement patterns during lameness evaluations. In conclusion, this study has shown that the use of continuous data provides additional information regarding gait adaptations to bilateral lameness that is complementary to the analysis of discrete variables. The main asset lies in the additional information regarding time dependence and duration of adaptations. This offers opportunities to identify functional adaptations during all phases of the stride cycle, instead of only during events related to peak values.

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6

Towards standardisation of surface electromyography measurements in the horse: bipolar electrode location

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Abstract

The use of surface electromyography in the field of animal locomotion has increased considerably over the past decade. However, no consensus exists on the methodology for data collection in horses. This study aimed to start the development of recommendations for bipolar electrode locations to collect surface electromyographic data from horses during dynamic tasks.

Data were collected from 21 superficial muscles of three horses during trot on a treadmill using linear electrode arrays. The data were assessed both quantitatively (signal-to-noise ratio (SNR) and coefficient of variation (CV)) and qualitatively (presence of crosstalk and activation patterns) to compare and select electrode locations for each muscle.

For most muscles and horses, the highest SNR values were detected near or cranial/proximal to the central region of the muscle. Concerning the CV, there were larger differences between muscles and horses than within muscles. Qualitatively, crosstalk was suspected to be present in the signals of twelve muscles but not in all locations in the arrays .

With this study, a first attempt is made to develop recommendations for bipolar electrode locations for muscle activity measurements during dynamic contractions in horses. The results may help to improve the reliability and reproducibility of study results in equine biomechanics.

Introduction

Surface electromyography (EMG), a non-invasive technique to assess muscle activity, has received an increasing interest in equine locomotion research. This aligns with the increased popularity of gait analysis used in equine biomechanics to detect and quantify lameness (i.e., pain during locomotion). In addition, several technical advances, mainly wireless measurement options, have enabled the use of this technique to evaluate animals during natural tasks. This can provide valuable insights into the neuromuscular system in both healthy and pathological conditions and allow for a better understanding of the complex biomechanics of animal locomotion.

Despite the technical advances in surface EMG, divergence in methodology between research groups has limited the comparability and repeatability of study results. In an attempt to standardise the methodology used to study muscle activity in humans, the SENIAM (Surface Electromyography for the Non-Invasive Assessment of Muscles) project[1], the International Society of Electromyography and Kinesiology (ISEK) guidelines[2] and more recently, the Consensus for Experimental Design in Electromyography (CEDE) project[3,4] have been established. These projects provide considerations for bipolar (SENIAM) and multichannel (CEDE) data collection, signal processing (CEDE) and how to report the results (CEDE, ISEK guidelines), improving the repeatability and reliability of human studies. This has resulted in a large, more reliable body of evidence on human locomotion.

Obtaining a representative and robust estimation of muscle activity is essential in fundamental veterinary research, rehabilitation and sports applications. In human muscles, though not expected to be different from equine muscles, the detected signal is known to be affected by the detection system (e.g. electrode size and position[1,5–7]), by the type of contraction (either dynamic or static)[5,8–10] and other factors affecting the recording of surface EMGs in general such as muscle architecture, skin preparation and adipose tissue[5,6]. Using surface EMG in horses, or animals in general, poses additional challenges compared to using this technique in humans. Examples of such challenges were outlined by Valentin and Zsoldos, which include but are not limited to: (1) how to prepare densely hairy and greasy skin in order to ensure electrode adhesion and to minimise skin impedance; (2) behavioural constraints, such as the impossibilities or difficulties to acquire a maximal voluntary contraction for normalisation purposes, to repeat specific movements, or to perform isometric contractions; and (3) electrode positioning on the muscles of interest, specifically in animals with large muscles such as the horse.[11] Among these, the position of the detection electrodes seems an essential yet-to-be-solved issue in equine studies.

It is commonly indicated that surface EMGs should be detected over the muscle belly, midway between the tendinous areas and the innervation zone.[1,12,13] Furthermore, electrode pairs should be placed in line with the muscle fibre direction, avoiding muscle borders, especially when studying dynamic contractions.[1,14] This would result in an optimal position for a pair of bipolar detection electrodes where the signal-to-noise ratio (SNR) is high, stride-to-stride variability is low, and the signal is most representative of the muscle.

This study aimed to start the development of recommendations for bipolar electrode locations to measure muscle activation in horses. These recommendations are meant for the group of users of surface EMG who focus their efforts on the equine locomotor apparatus. We used linear arrays of surface electrodes to investigate the effect of electrode location on surface EMGs in horses. The scope is limited to 21 superficial muscles and three Warmblood horses and should be considered as a starting point in our effort to improve the reproducibility of surface EMG study results.

Materials and methods

Horses

A convenience sample of three Dutch Warmblood horses (range values: age 12 – 14 years; body mass 545 – 611 kg; female) was included in this study. The horses were deemed sound by an experienced veterinarian (FSB) and had no known background with neurological disorders. The horses were regularly exercised and housed in boxes with daily turnout. All horses were exercised before the data collection period to allow acclimatisation to the treadmill [15]. Procedures were approved by the institutional animal welfare committee in compliance with the Dutch Act on Animal Experimentation.

Experimental protocol

The side of the horse on which the muscles were measured was randomly determined. The week before data collection, the horses were clipped, and ultrasound scans (Ultrasound machine: Epic 5, Philips, Eindhoven, The Netherlands; Transducer: L12-3 en C5-1 in musculoskeletal general pre-set) were performed on the selected muscles by a specialist from the European College of Veterinary Diagnostic Imaging (TR). This procedure allowed us to locate the muscle borders, tendinous areas and muscle fibre direction, which were marked on the skin by shaving thin lines.

Each horse went through six trials, with a maximum of two per day, where a different set of muscles were measured during each trial. For each trial, horses were exercised on a treadmill (Mustang 2200, Kagra AG, Fahrwangen, Switzerland) and underwent a 5-minute warm-up period at walk (6.0 – 6.5 km/h). Afterwards, the transition to trot was made (12.5 – 13.5 km/h, depending on individual gait stability). When the horse reached a stable trot, the measurement was started and data were collected for 60 seconds per trial.

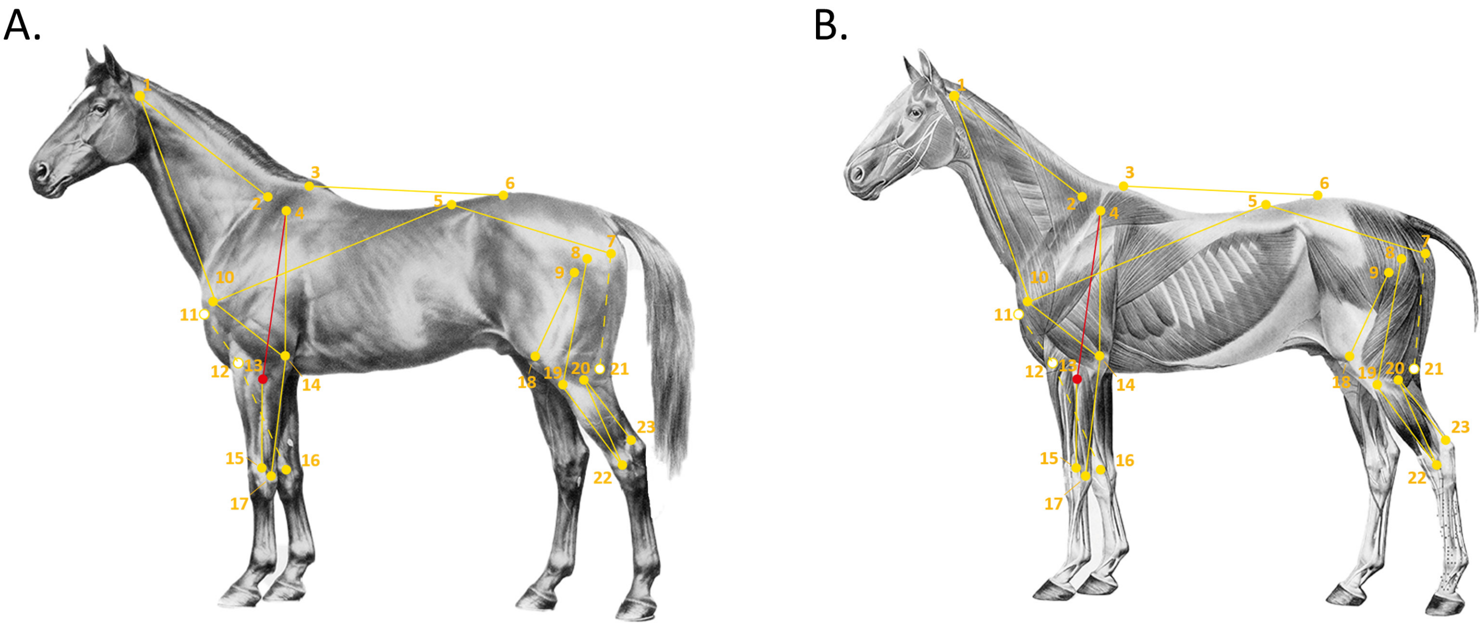


Figure 1. Anatomical landmarks used for the muscles studied. Panel A shows the landmarks and reference lines used from the skin surface, panel B over the muscles. The numbers indicate the anatomical landmarks as follows: (1) wing of the atlas; (2) cranial angle of the scapula; (3) withers, T6; (4) proximal tip of the spina scapula; (5) proc. spinosus of lumbar vertebra 4; (6) tubera sacrale; (7) tuber ischiadicus; (8) caudal part of the greater trochanter of the femur; (9) cranial part of the greater trochanter of the femur; (10) cranial part of the greater tuberosity of the humerus; (11) manubrium of the sternum; (12) lacerus fibrosus; (13) lateral side of the caput radii/tuberositas radialis; (14) olecranon; (15) lateral styloid process of the radius - ventral side; (16) medial styloid process of the radius; (17) lateral styloid process of the radius - caudal side; (18) tibial crest; (19) lateral tibial condyle and head of the fibula (20) lateral tibial condyle and head of the fibula; (21) at the height of the insertion of the m. gracilis; (22) lateral ridge of trochlea of talus; (23) calcaneal tubor. The lines represent the reference lines used for all muscles included in this study. The one red line (between anatomical landmarks 4 and 13) represents a reference line that was chosen incorrectly for the deltoideus muscle. The illustrations were adapted from 'Handbuch der Anatomie der Tiere für Künstler' (Ellenberger and Davis, 2013).

Electrode placement and data recording

For each muscle, specific anatomical landmarks were selected to aid in the placement of the electrodes. Specifically, two anatomical landmarks on opposite sides of the muscle were chosen, such that a straight line between them would parallel the muscle fibre direction, and the line would fall approximately equidistant between both marked muscle borders. The distance between these anatomical landmarks was measured using a standard measuring tape and rounded to half centimetres. Then, the 50% point between these anatomical landmarks was marked for each muscle. Care was taken that the horse was standing square with a neutral head and neck position for this whole procedure. An overview of the anatomical landmarks chosen for each muscle is shown in figure 1.

Prior to applying the electrodes, the skin was clipped (0.5 mm hair length) and thoroughly cleaned (ethanol 80%). Electrodes (pre-gelled Ag/AgCl; 3 mm diameter electrode; 24 mm inter-electrode distance; Cardinal Health, Waukegan, USA) were arranged into an array with a length depending on muscle size. Care was taken to ensure that two electrodes were positioned on either side of the 50% mark. When this was not possible, one electrode was placed on the 50% mark. Then, electrodes were placed adjacent to each other, along the muscle fibre direction, until we reached the muscle borders or the tendinous areas we determined with ultrasound scans. The ground electrode was positioned over either the tip of the spina scapula or the tuber coxae on the measured side. Tiny drops of mastix-based skin glue were used to fix the electrodes on the skin.

Surface EMG data were collected in monopolar derivation (referred to the average reference) and pre-amplified, sampled at 4000 Hz and A/D converted with 24-bit resolution (SAGA, TMSi, Oldenzaal, the Netherlands). In addition, kinematic data were collected using ten inertial measurement units (IMU; ProMove-Mini, Inertia Technology B.V., Enschede, The Netherlands) sampled at 200 Hz, to allow segmentation of strides. The IMUs were placed on the head, withers, pelvis, tubera coxae, and the lateral aspects of the four lower limbs of the horse. An additional IMU was mounted on the surface EMG equipment, which also has an embedded three-dimensional accelerometer (4000Hz), in order to synchronise the surface EMG and kinematic data.

Data analysis

All data analyses were performed in MATLAB (2022b, MathWorks, Natick, USA), and an overview of the data analysis procedure is illustrated in figure 2.

Surface EMG and kinematic signals were synchronised using a cross-correlation algorithm based on the dimensionless Euclidean norm of the 3D acceleration signals of both measurement systems.

Muscle	Proximal/Cranial	Distal/Caudal	Additional information	Safe zone per horse			Common safe zone
				HORSE 1	HORSE 2	HORSE 3	
Biceps Femoris	caudal part of the greater trochanter of the femur	lateral tibial condyle and head of the fibula		24% - 50%	19% - 45.5%	15.5% - 37%	24% - 37%
Brachiocephalicus	wing of the atlas	caudal part of the greater tuberosity of the humerus		47.5% - 60%	32.5% - 42.5% | 50% - 57.5%	41% - 64.5%	50% - 57.5%
Deltoideus	tip of the spina scapula	lateral side of the caput radii/ tuberositas radialis					
Extensor Carpi Communis	lateral tuberosity of the radius	lateral styloid process of the radius - ventral side		27 % - 50%	30% - 47%	12% - 43.5%	30% - 43.5%
Extensor Carpi Radialis	lacerus fibrosus	medial styloid process of the radius		28.5% - 47%	53% - 72%	39% - 50%	none
Extensor Digitorum Lateralis	lateral tibial condyle and head of the fibula	lateral ridge of trochlea of talus		22% - 50%	26% - 53.5%	20% - 32%	26% - 32%
Extensor Digitorum Longus	tibial tuberosity	lateral ridge of trochlea of talus		30-% - 50%	42% - 53%	36% - 71%	42% - 50%
Flexor Carpi Ulnaris	lateral tuberosity of the radius	accessory carpal bone		31.5% - 42%	42.5% - 52.5%	39% - 55.5%	39% - 42%
Flexor Digitorum Profundus	lateral tibial condyle and head of the fibula	calcaneal tubor		43.5% - 63%	26.5% - 53.5%	62.5% - 68.5%	none
Gluteus Medius	processus spinosus of L4	tuber ischiadicum			17.5% - 35% | 50% 56%	32% - 50%	32% - 35%
Latissimus dorsi	processus spinosus L4	cranial part of the greater tuberosity of the humerus	only one horse		41% - 48%		
Longissimus Dorsi	start of mane/top of the withers	between the tuber sacrale	6 cm out of the midline of the horse (processus spinosus)	40.5% - 57%	41.5% - 61.5%	45% - 62%	45% - 57%
Pectoralis pars Decendens	manubrium of the sternum	lacertus fibrosus		18% - 39.5%	39% - 58%	34.5% - 65%	39% - 39.5%
Petoralis pars Ascendens	olecranon	no caudal landmark	3 finger widths out of the midline of the horse. Distance indicated in cm from cranial landmark.	2.4 cm - 4.8 cm| 9.6 cm - 12.0 cm caudal of the olecranon	2.4 cm - 7.2 cm caudal of the olecranon		2.4 cm - 4.8 cm caudal of the olecranon
Rectus Abdominus	no cranial landmark	umbilicus	3 finger widths out of the midline of the horse. Distance indicated in cm from caudal landmark.	2.4 cm - 19.2 cm cranial from the umbilicus	2.4 cm - 19.2 cm cranial from the umbilicus	2.4 cm -16.8 cm cranial from the umbilicus	2.4 cm - 16.8 cm cranial from the umbilicus
Semitendinosus	tuber ischiadicum	at the height of the insertion of the gracilis		27.5% - 50%	26% - 56%	44% - 68%	27.5% - 50%
Splenius	wing of the atlas	cranial angle of the scapula		31% - 40.5% | 56.5% - 62.5%	51.5% - 61.5%	34% - 59.5%	56.5% - 59.5%
Triceps Brachi Caput Laterale	cranial part of the greater tuberosity of the humerus	olecranon		50% - 67.5%	56% - 68%	38% - 68%	56% - 67.5%
Triceps Brachi caput Longus	tip of the spina scapula	olecranon		36% - 53.5%	43% - 64.5%	43.5% - 53.5%	43.5% - 53.5%
Ulnaris Lateralis	lateral tuberosity of the radius	lateral styloid process of the radius - caudal side		36.5% - 53%	18% - 23.5% | 35.5% - 47%	31% - 62.5%	36.5% - 47%
Vastus Lateralis	greater trochanter of the femur	tibial crest		45% - 55%	31% - 50%	45% - 60%	45% - 50%

Table 1. Anatomical landmarks used for electrode placement. Safe zones are presented as the percentage (%) between anatomical landmarks measured from the proximal or cranial landmark. For the m. rectus abdominus and m. pectoralis pars descendens the distance is given in centimetres (cm). All channels for the m. deltoideus were excluded, therefore no safe zones are indicated for this muscle.

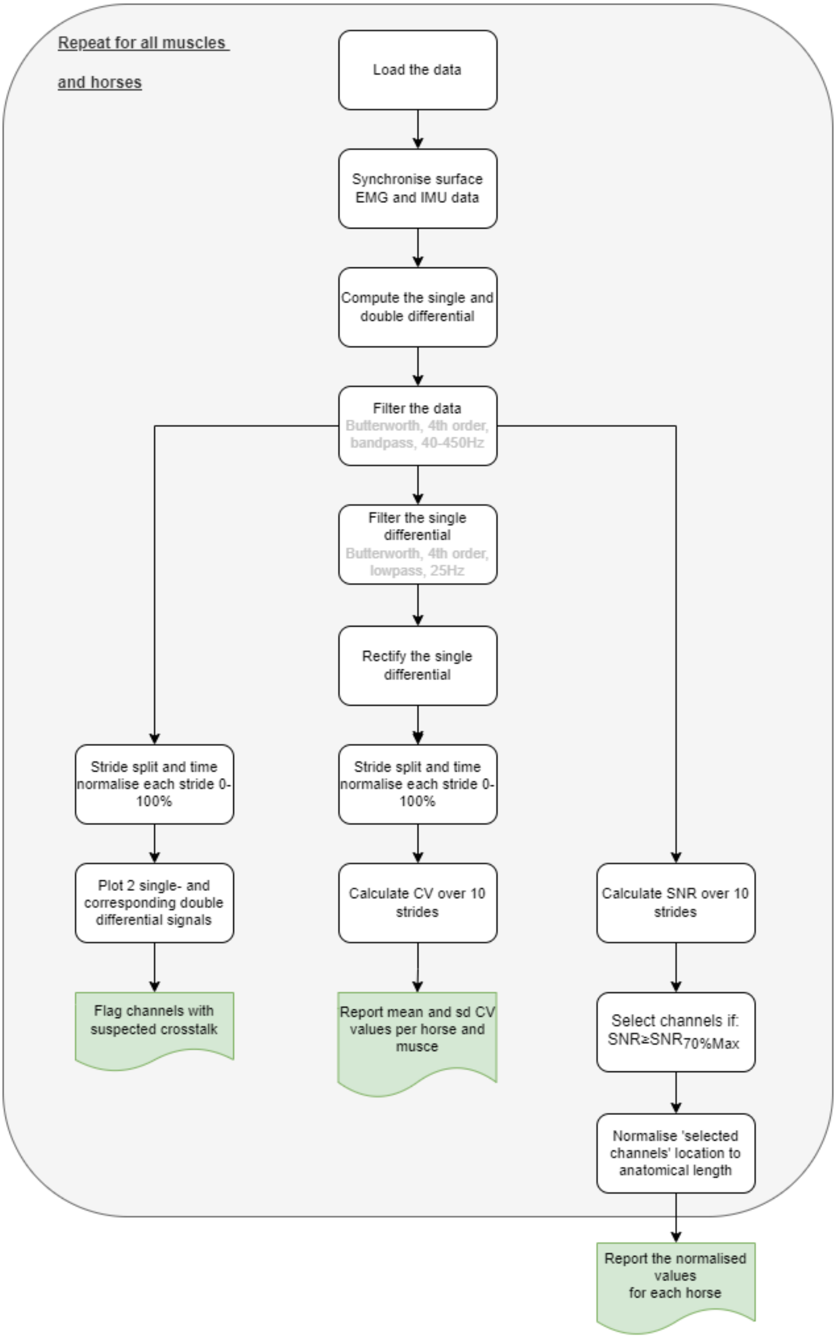


Figure 2. Flow diagram of the data analysis. This flow diagram illustrates the steps outlined in the data analysis section. Rectangle blocks represent the individual steps and green blocks indicate outputs. All steps within the grey rectangle were repeated for all muscles and horses. Electromyography (EMG); inertial measurement unit (IMU); coefficient of variation (CV); signal-to-noise ratio (SNR).

First, channels with extreme values were removed [16]. Then, single-differential surface EMG signals were computed in the proximal-distal or cranial-caudal direction as the difference between two monopolar signals recorded from adjacent electrodes. Double-differential signals were computed similarly from the single-differential signals. The single- and double-differential signals were bandpass filtered with a 4th order Butterworth filter (zero-lag; 40- 450 Hz cut-off frequencies [17].

To determine if some activation bursts may have originated from sources other than the targeted muscle (i.e., crosstalk), double- and single-differential signals were stride segmented and time-normalised from measured-side hind hoof impact to the following hoof impact from the same limb. Subsequently the double-differential and two single-differentials were plotted and qualitatively compared[18]. When an additional burst was visible in one or both of the single-differential signals, but not in the double-differential signal, these single-differential channels were classified as channels with suspected crosstalk.

For each single-differential signal, the signal-to-noise ratio (SNR) and coefficient of variation (CV) were computed over ten strides[20]. For the SNR computation, for each muscle and horse, ten segments of noise (no muscle activity) and signals (activity burst) were manually selected by an experienced evaluator (IHS, for an example, see supplementary figure 1) for all single-differential signals of a muscle simultaneously. The signal-to-noise ratio in dB was then approximated as follows:

$$SNR_{i,n} = 10 * log_{10} \frac{rms(signal\ segment_{i,n})^2}{rms(noise\ segment_{i,n})^2}$$

Where ί is the ίth extracted segment and η the ηth channel. The ten SNR values were then averaged to obtain one value per channel of each muscle and horse. Finally, single-differential channels in the array with SNR greater than or equal to 70% of the maximum value were considered for further analysis and are hereafter called the 'selected channels'.

For the CV computation, the single-differential signals were low pass filtered (4th order Butterworth filter, zero-lag; 25 Hz cut-off frequency), rectified and time-normalised from measured-side hind hoof impact to the following hoof impact of the same limb. After that, the mean and standard deviation were calculated for each point of the time-normalised stride. Then, the CV was calculated for each channel as follows:

$$CV_n(\%) = \frac{\sum_{k=1}^{k=101} \sigma_n}{\sum_{k=1}^{k=101} \mu_n} * 100$$

Where σ the standard deviation (sd) and μ the mean over the extracted normalised strides, k is the kth datapoint of the normalised strides, η is the ηth single-differential channel. The mean CV was calculated for each single-differential channel by averaging the CV values per channel over each stride. This provided *n* values for each muscle (where *n* is equal to the length of the electrode array -1), which describe the distribution CV over the array per muscle per horse. The CV was evaluated to assess the within muscle repeatability and is expressed in percentage

(%). In addition, in order to compare with other studies, we computed the CV with the method proposed by Winter et al. [19] and the variance ratio [21] on both the non-normalised and amplitude normalised surface EMG signals [22]. These results can be found in supplementary table 1.

Safe zone determination

To control for between-subject variability in muscle size, the longitudinal locations of the electrodes were normalised to the distance between the anatomical landmarks used for electrode placement. Then, the normalised locations of the selected channels were calculated for all horses and muscles. Adjacent selected channels were considered as a 'safe zone' for each individual muscle. The proximal/cranial and distal/caudal extends of these zones were reported, where we allowed for the possibility of having multiple of these zones within a muscle. In order to determine the common safe zone between horses, the overlap of the safe zones of the individual horses was reported.

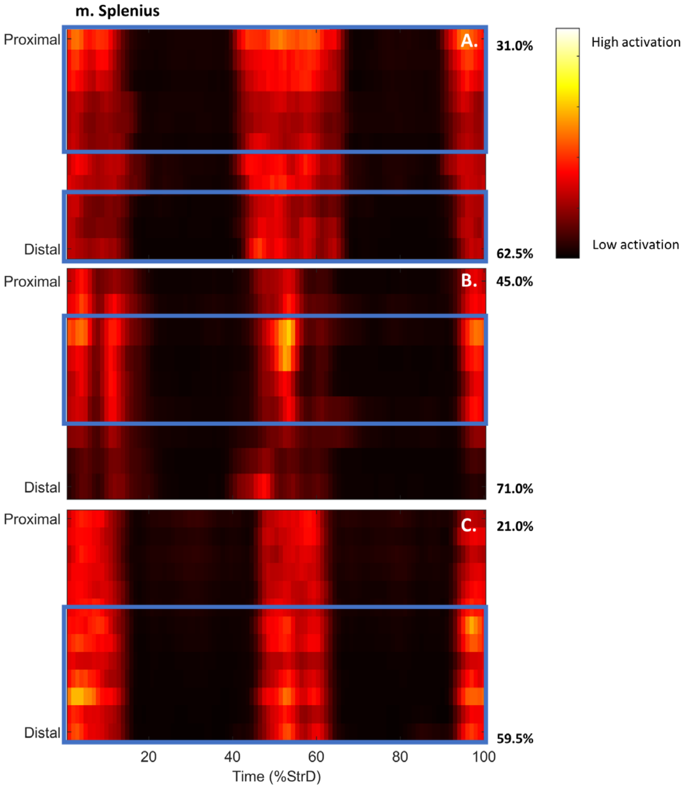


Figure 3. An example of the differences between horses with one or two overlapping safe zones for the m. splenius. This figure shows the activation pattern of the m. splenius over an averaged time-normalised stride for the three horses separately. Each row in a sub-panel represents the mean activation pattern from a channel. The top row shows the most proximal channel and the bottom row the most distal channel for that horse. On the right side of the panel the corresponding normalised location of that channel is given as a percentage between the anatomical landmarks used. The blue boxes indicate the selected channels. The colour scale gives an estimate of the amplitude, where the brighter colours represent the higher voltages. Panel A. shows the pattern of horse H01, panel B. of horse H02 and panel C. of horse H03.

Results

General description

All horses performed the six trials without difficulties (mean ±sd strides per trial = 76.8 ±4.7). In two horses, muscles were measured on their left side, in the other horse, on the right side. For the m. deltoideus, the chosen reference line was inappropriate (figure 1), therefore all channels were excluded for this muscle. For the final dataset, 27/449 channels were excluded due to extreme values (supplementary table 2).

		Horse	
Muscle	**HORSE 1**	**HORSE 2**	**HORSE 3**
Biceps Femoris	39.1 ± 2.8	43.2 ± 3.8	41.6 ± 8
Brachiocephalicus	44.6 ± 2.5	53 ± 6.8	41.8 ± 1.3
Extensor Carpi Communis	48.2 ± 4.2	44.6 ± 1.3	37.1 ± 3.6
Extensor Carpi Radialis	58.6 ± 4.9	50.7 ± 2.8	45.9 ± 4.1
Extensor Digitorum Lateralis	44.8 ± 2.9	36.1 ± 2	35.9 ± 4.2
Extensor Digitorum Longus	45.9 ± 4.5	40.4 ± 2.7	37.1 ± 3
Flexor Carpi Ulnaris	39.1 ± 1.8	41.9 ± 2.2	53.9 ± 5.5
Flexor Digitorum Profundus	42.6 ± 6.8	51.1 ± 6.4	32.8 ± 7.9
Gluteus Medius		48.5 ± 5.7	38.3 ± 7.3
Latissimus dorsi		30.2 ± 1.5	
Longissimus Dorsi	31.5 ± 2.2	50.9 ± 4.8	41.5 ± 3.8
Pectoralis pars Decendens	56.2 ± 7.2	49.7 ± 6.5	39.8 ± 4
Petoralis pars Ascendens	41.6 ± 12.3	40.3 ± 3.8	40.5 ± 2.2
Rectus Abdominus	71.1 ± 13.4	92.2 ± 2.5	48.8 ± 20.3
Semitendinosus	48 ± 18.9	56.4 ± 4.8	35.3 ± 2.1
Splenius	42.8 ± 3.1	43.2 ± 2.6	44.8 ± 3.2
Triceps Brachi caput Laterale	47.8 ± 2.5	50.3 ± 2.1	43.3 ± 7.2
Triceps Brachi caput Longus	34.4 ± 1.7	40.2 ± 1.1	37.8 ± 4.5
Ulnaris Lateralis	42.8 ± 2.3	42.9 ± 3.6	56.5 ± 5.2
Vastus Lateralis	41.1 ± 3	39.6 ± 3.3	43.2 ± 4.4

Table 2. Mean and standard deviation (sd) of the coefficient of variation (CV; %) per muscle and horse. The mean CV was calculated for each single-differential channel by averaging the CV values per channel over each stride. This provided n values for each muscle (where n is equal to the length of the electrode array -1), which describe the distribution CV over the array per muscle per horse.

Spatial localisation of the SNR and CV distribution

For most muscles and horses, the highest SNR values were found in the channels cranial or proximal to the 50% marks (table 1). There were clear differences between channels for most muscles in terms of SNR and the qualitative signal assessment (figure 3). Differences were observed between subjects for some muscles, mainly in the m. extensor carpi radialis and m. flexor digitorum profundus (table 1). For these muscles, there were no overlapping zones between horses where SNR values were equal to or larger than 70% of the maximum value (figure 4).

In general, CV values differed between locations, muscles and horses and varied between 29.3% and 96.0% with a mean ± sd of 44.8 ±10.1%. To give an impression of the differences within a muscle between horses, mean and sd per muscle and horse are given in table 2. In addition, the CV values, as well as the SNR values and the other measures of variability, for all tested locations and muscles were presented in supplementary table 1. A representative example of the differences within a muscle and between two horses is given in figure 5.

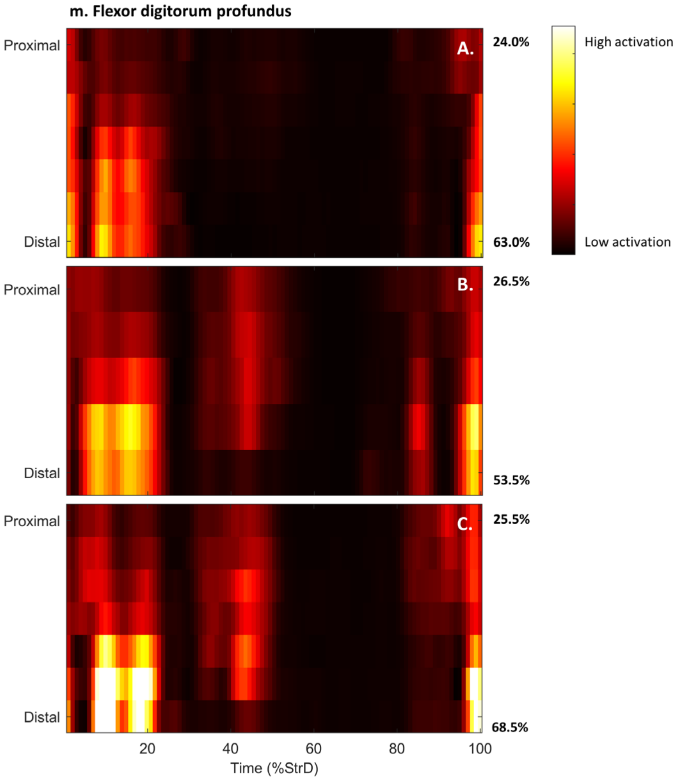


Figure 4. An example of the differences between horses with no overlapping safe zone. This figure shows the activation pattern of the m. flexor digitorum profundus over an averaged time-normalised stride for the three horses separately. Each row in a sub-panel represents the mean activation pattern from a channel. The top row shows the most proximal channel and the bottom row the most distal channel for that horse. On the right side of the panel the corresponding normalised location of that channel is given as a percentage between the anatomical landmarks used. The colour scale gives an estimate of the amplitude in µV, where the brighter colours represent the higher voltages. Panel A. shows the pattern of horse H01, panel B. of horse H02 and panel C. of horse H03.

Single- vs double differential signals

In a few signals from some muscles crosstalk was suspected. For three muscles, crosstalk was suspected for more than one horse. These were the m. Brachiocephalicus (for H02 and H03; figure 6), m. Semitendinosus (H01 and H02) and m. longissimus dorsi (H01 and H03). Other muscles where crosstalk was suspected were m. extensor digitorum lateralis and m. triceps brachii caput laterale for horse H01; m. extensor digitorum longus and m. latissimus dorsi for horse H02 and m. extensor carpi communis, m. pectoralis pars descendens and m. vastus lateralis for horse H03. For these muscles not all signals were suspected to contain crosstalk, only those from the channels at the ends of the arrays.

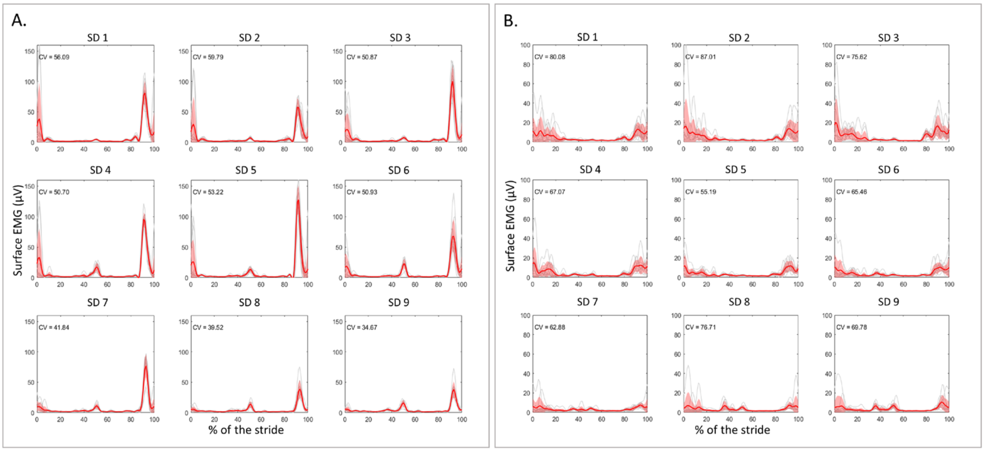
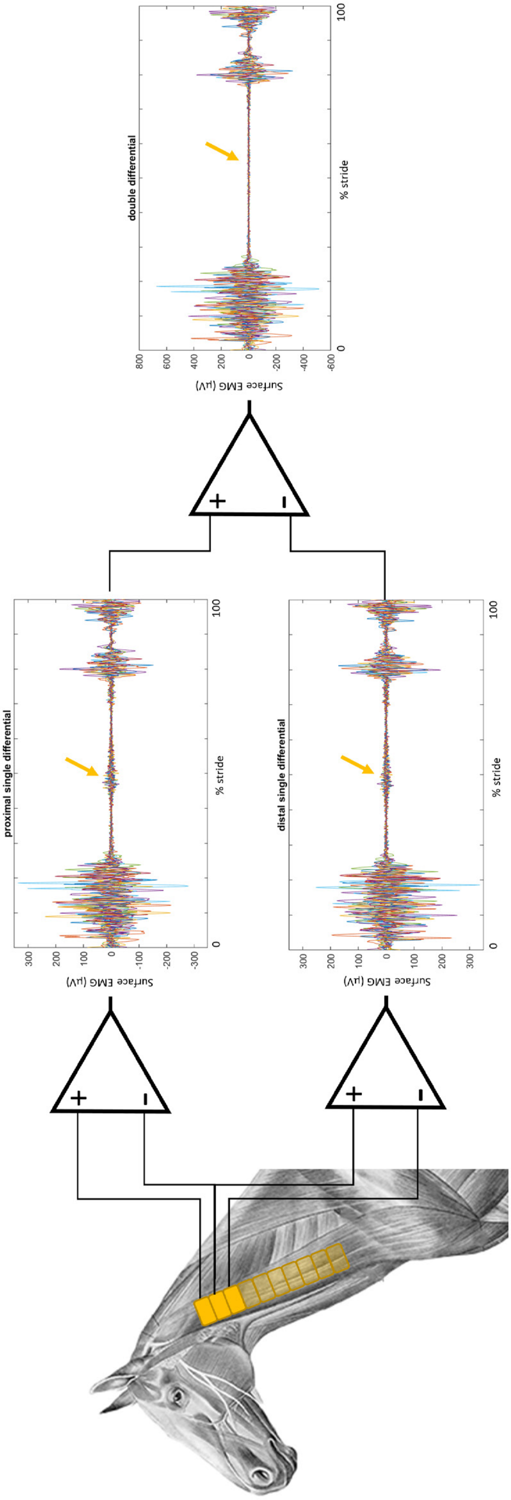


Figure 5. Two examples for the inter-muscle surface EMG profiles from the m. semitendinosus. This figure shows the mean (solid red) and standard deviation (shaded area) of the time-normalised (ten strides) surface EMG data (grey; in micro volts (μV)) of the m. semitendinosus of horse H01 (panel A) and H02 (panel B). Every subpanel shows the data of one location from the most proximal single differential (SD 1) to the most distal single differential (SD 9). The coefficient of variation (CV) in indicated for ever location.

Discussion

The challenge of choosing electrode locations for surface EMG recordings in horses is essential for many fields, ranging from basic research to clinical applications. Despite the increasing number of studies involving surface EMG in horses, there is a substantial discrepancy or unclarity regarding the used electrode locations. Here, we addressed this issue by investigating the signal quality for 21 muscles in three horses using electrode arrays. We demonstrated that, for most muscles and horses, the highest SNR values were detected near or cranial/proximal to the central region of the muscle. There were larger differences in CV values between muscles and horses than within muscles. The current results may provide initial recommendations for choosing electrode locations to collect surface EMG data from horses.

It is commonly indicated that to measure reliable surface EMG signals, detection electrodes need to be placed between the innervation zone and tendinous areas and during the motion the detection electrodes should not move over either of these zones [1,13,14,23,24]. In the current study, tendinous areas were determined using ultrasound scans. The innervation zones are usually identified by having the test subject perform isometric contractions while measured using grids or arrays of electrodes [13,25–27] or by electrical stimulation [28]. The maximal shift in innervation zone location during the motion cycle can then be determined by performing isometric contractions at the extremes of the motion cycle [7,24]. However, this is impossible when studying horses, as we cannot ask the subject to perform a specific task. We were limited to a locomotion task, in this case trotting, which was standardised in speed by having the


proximal single differential
Surface EMG (µV)
300
200
100
0
-100
-200
-300
0
100
% stride
distal single differential
Surface EMG (µV)
300
200
100
0
100
200
300
0
100
% stride
double differential
Surface EMG (µV)
800
600
400
200
0
-200
-400
-600
0
100
% stride
+
-

horses trot on a treadmill. It was therefore impossible to reliably quantify specific parameters indicative of the presence of innervation zones, especially since we do not know the degree of muscle lengthening and shortening during the gait cycle in the horse, and skin displacement can be substantial [29]. Therefore, we did not include any parameters indicative of innervation zones, and our considerations are solely based on SNR values within a muscle during trot.

The CV was used to evaluate the intra-location (within muscle and horse) reliability of the surface EMG profiles. Even though CV values did not greatly differ between the locations within most muscles, variability was still relatively high for some muscles, despite the horses being trotted at a stable speed on a treadmill. The variability was highest for bi-articular muscles, such as the m. semitendinosus or the m. triceps brachii caput longum, and the m. rectus abdominus. This was to be expected for the bi-articular muscles and in agreement with a recent study on the reliability and repeatability of surface EMG in horses [30] and during walking and running in humans [31–33]. In addition, the bi-articular muscles also showed larger differences between locations, as can be found in the supplementary material. We know there are different methods to calculate the CV, and the CV is sensitive to the method of amplitude normalisation. We did not amplitude normalise our data, and comparisons were only made within horse and muscle. Other measures such as the variance ratio which can be found in the supplementary table S1, may be worth investigating to assess the variability of surface EMG signals in horses. This measure is independent of which normalisation method is used [22] and the number of analysed strides [34]. The last being of high importance, as it may be challenging to get enough repetitions of the same task when working with animals.

Differences were subjectively observed between muscles in terms of the consistency of the activation pattern across the different channels. In voluntary dynamic contractions, as is the case during locomotion tasks, activity seems to be distributed more heterogeneously within pennate muscles in humans [35,36]. This may explain why, for more fusiform muscles, for instance, the m. brachiocephalicus, channels seemed very similar to each other in terms of activation pattern and timing, while for other more pennate muscles, for instance, the m. flexor digitorum profundus [37], the activation pattern varied more for different channels. This could mean that for pennate muscles, multiple electrode locations would need to be used when aiming to describe the whole muscle activation pattern.

For some muscles, there was no overlap in safe zones between horses. This might be due to the differences in innervation zone locations between individuals, as it is known that innervation zones differ in location in human muscles [7,12,38]. Furthermore, the muscles where no overlapping

◀ Figure 6. An example of the comparison between single and double differential signals with suspected crosstalk. This figure shows the time-normalised strides data from the m. brachocephalicus of horse H03. Panel A shows a schematic overview of the brachiocephalic muscle and the electrode array. The solid rectangles represent the electrodes from which the signals were compared. Panel B shows the time-normalised single differential signals. Panel C shows the time-normalised double differential signals derived from the single differential signals in panel B. The arrows indicate the activation burst that is suspected to be crosstalk. These bursts are visible in the single differential signals, but not in the double differential signals. Note that the squares do not represent actual electrode size.

safe zones were found were all distal limb muscles. These muscles are generally smaller in size, their architecture is more pennate, and they have longer tendons relative to the muscle size. Therefore, we could place arrays with fewer electrodes on these muscles; hence, it was not surprising to find smaller or no overlapping safe zones. In addition, due to conformational differences between horses, it was not always possible to place arrays with a similar length on each horse. For instance, on horse H01, we could not place electrodes further than the 53.5% point on the m. flexor digitorum profundus, where on the other horses we could place electrodes up to up to 63% and 68.5% of the distance between the anatomical landmarks. Still, the SNR was highest for the more distally located channels of H01, similar to the other two horses, where the normalised safe zones were also located more distally.

For this study, only a small and homogenous group of horses was included. Extrapolating our findings to the entire equine population must therefore be done with caution. Moreover, even in this small homogenous sample, large differences between horses were found for some muscles. It is known that innervation zone locations differ between humans [39], and the SENIAM recommendations do not always lead to the most optimal electrode position for all individuals [7]. This stresses the importance of choosing the correct electrode configuration to fit the aims of individual studies. Based on our findings, we propose that the bipolar electrode positions within the safe zones found in this study might be sufficient to determine muscle onset and offset during gait tasks. However, especially for non-parallel or fusiform muscles and questions related to activation distribution over a muscle, the proposed locations alone might not be sufficient and multiple sets of detection electrodes or grids might be required.

With this project, a first effort was made to standardise bipolar electrode locations in horses. The recommended locations of this study should be considered as a starting point and so far they only concern the locations for bipolar measurements during dynamic tasks. The recommendations provided will change over time as new empirical evidence emerges and new technologies to record surface EMGs are developed. Advances in technology, such as the development of new types of electrodes (e.g. high adhesion stretchable electrodes [40,41], screen-printed electrodes [42] or electrodes integrated in textiles [43,44]), will provide solutions that allow for approximation of muscle activation in the horse in a more natural way. We foresee that the recommendations must be updated accordingly. Further efforts must be made to continue the standardisation of surface EMG measurements in horses and other species. Examples are: (1) how to prepare greasy and densely hairy skin for reliable surface EMG recordings; (2) Where to place electrodes to measure muscles that were not included in this study or where to place grids for high-definition surface EMG measurements and (3) how to process the signals, for instance in terms of normalisation procedures to compare between individuals and the number of strides needed to draw reliable conclusions. This will require the combined effort of experts in the field, and we sincerely hope that the community will contribute to this in the near future.

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7

Open science

In this chapter, contributions made to open science are outlined. For the different chapters in this thesis, only specific parts of these datasets were used. Though we believe that much more meaningful information can be extracted. Three kinematic datasets and one kinematic and electromyographic dataset were, or are in preparation to be, published to support the chapters in this thesis. The are the following datasets: single-limb lameness (forelimb and hindlimb), bilateral lameness and muscle activity at trot. An overview of the conditions, gaits and techniques used in the different datasets can be found in figure 1.

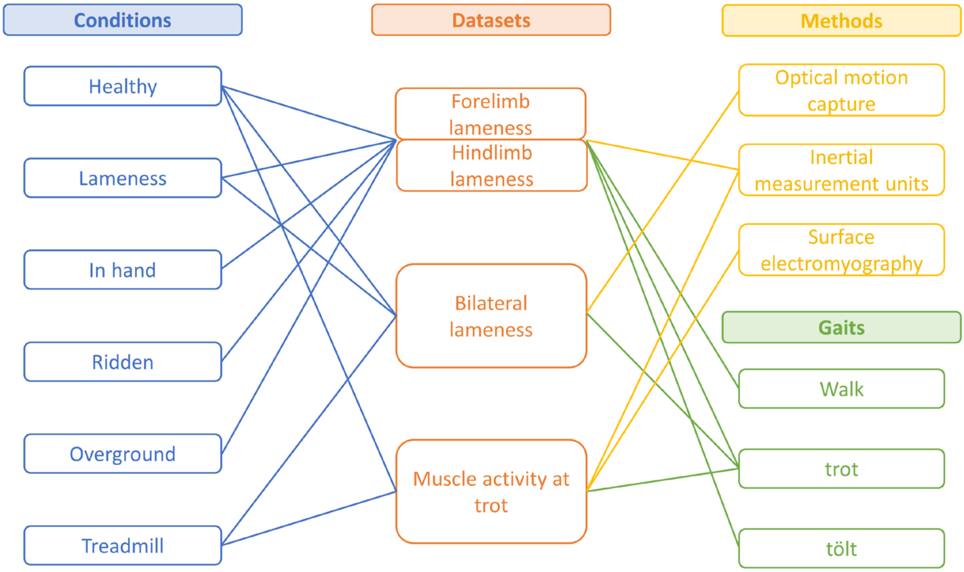

Figure 1. Overview of the contents of the datasets that support the results in this thesis.

Single-limb lameness

Forelimb lameness

The forelimb lameness dataset contains data of Icelandic horses performing walk and trot in hand and walk, trot and tölt (at different speeds) while ridden. These gaits and conditions were performed both in a 'healthy' state (no intervention) and after single-forelimb lameness induction using the shoe model[1] (figure 2, left). Fifteen inertial measurement units (IMUs), sampling at 500Hz, were attached to the horse and one on the rider (figure 2, right). The devices, among other sensors, contained low and high-g 3-axis accelerometers, gyroscopes, global positioning system sensor and magnetometer. The dataset consists of 39 individual datasets (n = 10 horses), where for each horse the following datasets are present: baseline in hand (n=10), baseline ridden (n=10), induction in hand (n=9) and induction ridden (n=10).

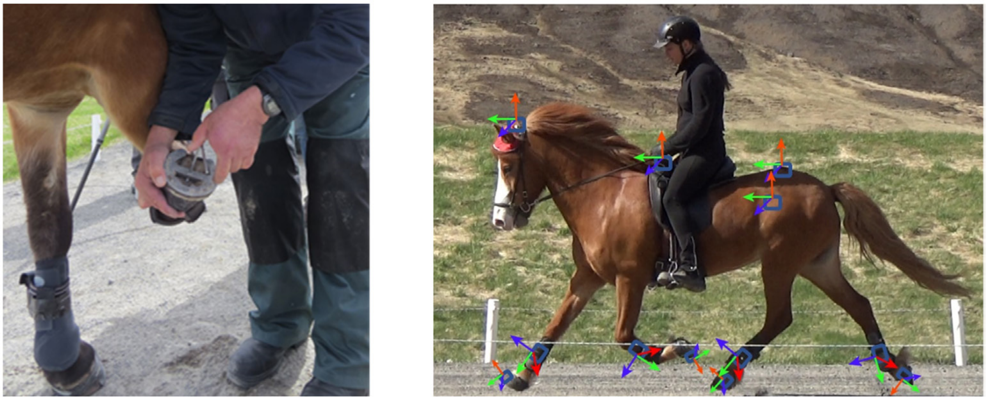

Figure 2. Images illustrating methodological aspects of the single-limb lameness in Icelandic horses datasets. Left: lameness induction using the sole pressure model. Right: Placement of the IMUs

Hindlimb lameness

The Hindlimb lameness dataset is very similar to the forelimb lameness dataset. Again, this dataset contains data of Icelandic horses performing walk and trot in hand and walk, trot and tölt while ridden. These gaits and conditions were performed both in a 'healthy' state (no intervention) and after single-hindlimb lameness induction using the shoe model[1] (figure 2, left). Fifteen inertial measurement units (IMUs), sampling at 500Hz, were attached to the horse and one on the rider in the same way as for the forelimb lameness dataset. The hindlimb lameness dataset consists of 40 individual datasets (n = 11 horses), where for each horse the following datasets are present: baseline in hand (n=11), baseline ridden (n=9), induction in hand (n=10) and induction ridden (n=10).

Bilateral lameness

The bilateral lameness dataset consists of sixteen Shetland ponies performing trot on a treadmill at 3 different timepoints (before, 3 months after and 6 months after surgical intervention[2]). Twenty-eight reflective markers were placed at relevant anatomical landmarks (figure 3) and

Figure 3. One of the study subjects, equipped with 28 reflective markers, at trot. The markers used for the analysis were: poll, withers, dorsal spinal process of T15, tuber sacrale, left/right tuber coxae, left/right spina scapulae, left/right greater tubercle of the humerus, left/right lateral aspect of the carpus, left/right- front/hind lateral aspect of the fetlock, left/right- front/hind lateral aspect of the proximal dorsal hoof wall, left/right trochanter major of the femur and left/right lateral aspect of the tarsus.

six optical motion capture cameras were used to capture the position of these markers in space at 200Hz. In addition, videos in the sagittal plane of the horse were recorded at 30Hz using a standard camera. Both the raw and processed data are shared, where the processed data contains discrete values calculated over single strides and stride-time normalised continuous data.

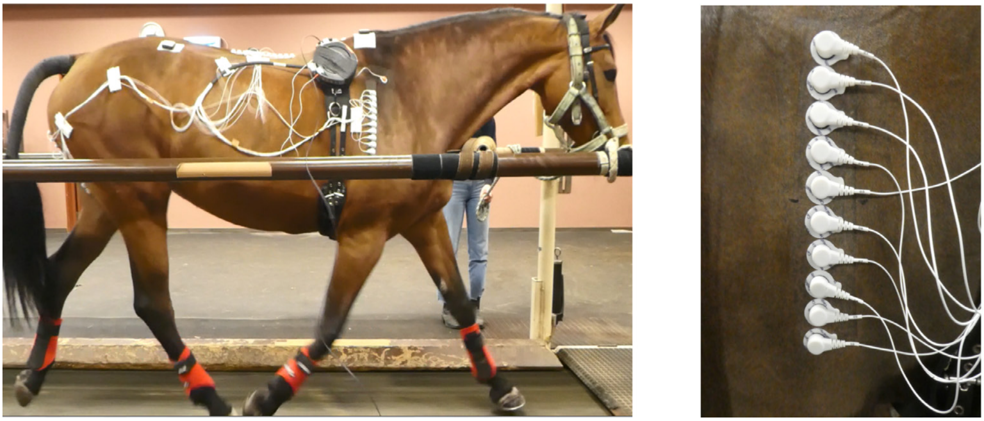

Figure 4. Images illustrating methodological aspects of the muscle activity at trot dataset. Left: an overview of one of the horses equipped for one measurement session. Arrays of electrodes are placed over the m. semitendinosus, m. longissimus dorsi and m. triceps brachii. Inertial measurement units (IMUs) are attached to the poll, withers, tubera sacrale, left and right tuber coxae and the lateral aspects of the cannon-bone of all four limbs. In addition, one IMU is placed on top of the surface electromyography (EMG) equipment for synchronisation purposes. Right: an example of an electrode array of ten electrodes over one muscle.

Muscle activity at trot

The muscle activity at trot dataset contains data of three horses performing six sessions of trot on a treadmill. For two horses, 19 muscles and for one horse 21 muscles were measured using surface electromyography at 4000Hz and data collection per session lasted for 60 seconds. In addition, kinematic data were simultaneously collected using ten IMUs (figure 4, left). For each muscle, several locations were measured using linear electrode arrays (figure 4, right) in the direction of the muscle fibres, with an inter-electrode distance of 24mm. The size of the electrode array, and thus the amount of data collected per muscle within horse, depended on the available skin surface over a muscle. Both the raw electromyographic and kinematic data are shared, as well as the footfall timing for the hindlimb impacts used for stride splitting.

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8

General discussion

There is a need for a more comprehensive approach in equine locomotion research to aid our understanding of how a horse moves. Existing techniques should be applied in a broader context, and the methodological toolbox to quantify locomotion needs to be expanded to achieve this goal. This final chapter starts with a summary of the main results of this thesis. Then, the scientific approach for the work in this thesis will be discussed in the context of whether locomotion in research should reflect locomotion in the real-world environment. This will be followed by thoughts on how a more comprehensive approach could contribute to a better understanding of equine locomotion. The chapter concludes with a future perspective on equine locomotion research from a scientific and societal point of view.

Summary of the main findings

Chapter 2 describes an observational study with 65 horses on the differences between breeds and gaits in terms of upper body vertical movement and temporal stride parameters. Three different breeds (Warmblood, Iberian and Icelandic horses) and five gaits (walk and trot for all horses, tölt and pace additionally for Icelandic horses) were assessed. The primary outcome parameters were the upper body's vertical range of motion (ROMz), timing of the local vertical displacement minima and maxima, and stride, stance, and swing duration.

The results showed that the smaller the breed, the smaller the upper-body ROMz and the shorter the stride, stance and swing duration. This may be a logical consequence of having shorter limbs. The timing of vertical displacement minima and maxima could be explained by walking and running mechanics. In walk, vertical displacement minima of the withers and pelvis were closely related to the moment when the two contralateral limbs were both in contact with the ground; thus the moment the two fore- or hindlimbs were in maximal spread. The timings of the vertical displacement maxima were related to the midstance of the fore- and hindlimbs. For running gaits (trot, tölt and pace), the vertical displacement minima were closely related in time with the moment of midstance, whereas the maxima were reached around the push-off phase.

Chapters 3 and 4 describe two experimental studies in which lameness was induced in a forelimb in ten Icelandic horses (**chapter 3**) and in a hindlimb in eleven Icelandic horses (**chapter 4**). The aim was to describe the kinematic changes, both spatial and temporal, related to this induced lameness during walk, trot and tölt. The data were collected under field conditions, both in hand (walk and trot) and while the horses were ridden (walk, trot, and tölt). Mainly upper-body movement asymmetry and interlimb timing were assessed.

These studies revealed that 'traditional' lameness metrics (i.e., upper-body asymmetry values) indicate fore- and hindlimb lameness at walk and trot in Icelandic horses. At the tölt, these metrics could also discriminate between normal and hindlimb lameness conditions, but not as well for forelimb lameness. In addition, Icelandic horses move less symmetrically at tölt than at walk and trot, even without lameness induction. This makes it more challenging to differentiate between sound and lame conditions based on upper-body asymmetry at the tölt. Regarding interlimb timing, lameness reduces the relative time (as a percentage of the stride duration) from the sound to the contralateral limb impact at all gaits but not vice versa. Interlimb timing may thus be a valuable parameter to discriminate between normal and pathological conditions.

Chapter 5 describes an experimental study where differences were assessed between continuous and discrete analysis methods applied to data from long-term bilateral hindlimb lameness in sixteen Shetland ponies. These ponies were enrolled in a cartilage repair study, and gait analysis was used as a clinical outcome parameter. Full-body kinematics were captured using 3D optical motion capture while the ponies were trotted on a treadmill.

Our results show that continuous data analysis may benefit the evaluation of bilaterally lame ponies, as these do not become asymmetrical in terms of upper-body vertical movement. The two methods are complementary: discrete data gives easy-to-interpret values, and continuous data provides additional information about the timing and duration of the changes. In addition, continuous data analysis may reveal kinematic adaptations that occur outside of events related to peak values (often described well using discrete values). The main changes in movement were observed in the pelvis rotations (both pitch and roll), and in interlimb timing.

Chapter 6 describes an observational study into the effect of electrode location on equine locomotor muscle activity measurements when using surface electromyography (EMG). The aim was to develop recommendations on electrode location for collecting surface EMG data in horses. Surface EMG data was collected from twenty muscles in three Warmblood horses trotting on a treadmill. The primary outcome parameter was signal-to-noise ratio (SNR), and locations were compared within muscle and horse.

Based on the outcomes of this study, electrode locations were proposed for sixteen out of twenty muscles in Warmblood horses. These results may help to standardise methodologies across research groups. We believe this may benefit the field and aid in creating a large and reliable body of evidence on muscle activation in moving horses. The standardisation work is unfinished, and the recommendations may change with new emerging technologies.

In **Chapter 7**, the datasets used for chapters 3, 4, 5, and 6 in this thesis are shared (i.e. the datasets of the fore- and hindlimb lameness induction studies, the bilateral lameness study, and the dataset used to determine electrode location for surface EMG measurements). With this chapter we aim to advocate open science by sharing the data supporting our findings.

To what extent should locomotion in research reflect locomotion in the real-world environment?

The general goal of locomotion analysis is to obtain insights into motor function or, in clinical cases, insights into underlying impairments that are responsible for decreased motor functioning. To obtain these insights, it can be argued that the environment and setting of locomotion analysis should resemble the real-world environment of the subject to be studied. For many horses, this includes settings where the horse must turn, speed up or slow down in response to dynamic situations. In contrast, locomotion analysis is usually conducted in the standardised environment of a laboratory and standardised models are used to mimic lameness. This gives rise to discrepancies, as the analysed movements may not always be comparable to movements shown in daily life This highlights the impact of methodological choices and stresses that the measurement protocol should be suitable to address the underlying question. In biomechanics research, especially when working with non-human animals, scientists always need to balance ecological validity, data integrity, and reliability. To which side the scale tips mainly depends on the research questions. As such, ecological validity might weigh heavier for applied research questions, whereas for fundamental or methodological research, we might emphasise ensuring the highest possible data integrity and reliability. These considerations affected our methodological choices when designing the studies presented in this thesis.

Reflections on the methodologies used in this thesis

To come closer to studying ecologically valid locomotion, we deviated from standard practice in equine locomotion research in one way or another in all chapters of this thesis.

For **chapters 2, 3 and 4** we moved out of the laboratory setting, and we showed that reliable data can be collected from horses, both healthy and lame, in field conditions. Using IMUs instead of optical motion capture has brought new opportunities that allow for data collection outside of a laboratory setting, potentially increasing ecological validity. IMUs have proven to be reliable and accurate tools to capture equine locomotion.[1,2] They hold significant advantages over optical motion capture, being portable and suitable for use under any external light condition. This helps the transition from the laboratory into the field, allowing for data to be collected from more natural locomotion and thereby increasing the ecological validity. In addition, in **chapter 2**, different breeds were compared, the outcome of which emphasised that we should evaluate an individual in the context of its own breed, not of the wider general population of horses. In **chapters 3 and 4**, we assessed the effects of acute lameness at gaits other than trot.

For **chapters 5 and 6**, the scale tipped towards the use of a strictly controlled laboratory setting. We used the treadmill to standardise measurements over time. For **chapter 5**, where we evaluated bilateral lameness and measured long-term effects; this allowed us to attribute observed changes over time to the applied intervention rather than to differences in the experimental setting. Since the measurements were conducted with 3-month intervals, it would have been difficult to reproduce the same experimental setting for each time point. This is especially important regarding speed, since we know that speed influences parameters used to evaluate lameness.[3] The research described in **chapter 6,** in which we described the use of surface EMG in horses, was conducted with the aim to improve reliability and especially repeatability of studies of muscle function in horses. We have shown that the longitudinal positioning of the surface EMG sensors over the muscle can influence signal quality, and even the measured activation patterns, due to crosstalk. Therefore, standardising electrode locations within the horse (between sides or timepoints), between horses, and between research groups is highly important to obtain valid data, draw valid conclusions, and compare results. Surface EMG is a relatively new method to the equine field and brings an exciting new approach to studying movement in horses. However, before this tool can be widely implemented in locomotion research, a few additional hurdles need to be overcome. As such, little is known about the electrode-skin interface when working with hairy and greasy animals such as horses. This interface, however, is essential for collecting reliable data. In addition, there is no consensus on how to treat the collected data, except for filtering recommendations[4], which makes it difficult to compare results between research groups. Lastly, the interpretation of the data is not as intuitive as for instance the interpretation of kinematic data. This may be further complicated by the fact that small changes in measurement protocols or circumstances (i.e. electrode location, task performance or environmental factors) can have a large effect on the collected data.

When we can standardise, should we always?

A common method to standardise equine locomotion measurements is using a treadmill to control for direction and speed. Locomotion was measured overground for **chapters 2, 3, and 4**, whereas for **chapters 5 and 6**, the treadmill was used. Studies performed on human subjects have confirmed that the kinematic, kinetic and muscle activation representations of treadmill walking and running show very high similarities, but are not equivalent to overground walking[5,6] and running[7,8]. Besides mean stride-based differences, general variability between strides is decreased when moving on a treadmill.[8] This is logical, given the lack of acceleration, deceleration or other disturbances. For horses, it is known that they show an increased relative stance duration, increased retraction and decreased vertical movement of the hooves and withers when trotting on a treadmill compared to overground.[9,10] The aspects considered above regarding differences between treadmill and overground locomotion, imply that one mode may not simply replace the other and that caution is warranted when generalising results across conditions.

A common method to study lameness in equine locomotion is to use a lameness model. Since the introduction of the low-risk, highly repeatable and completely reversible shoe model[11], many studies have focussed on the effects of acute, single-limb lameness at trot[12]. However, lameness can have many different causes, many unrelated to hoof pain and is probably more often chronic than acute. One of the strong points of this thesis is that we did not only look at lameness induced using the shoe model (**chapters 3 and 4**), but also investigated a relatively large group of sixteen ponies with lameness due to bilateral osteochondral defects in the medial epicondyle of the femur (**chapter 5**), which we followed up until six months after intervention. Even though these induced osteochondral defects may be an uncommon cause of lameness, the results may still well reflect long-term adaptations to hindlimb lameness. This holds the potential to aid the understanding of movement chain adaptations related to long-term decreased loading of a limb or limbs, which cannot be studied using acute lameness models. The use of the shoe model has brought us a strong understanding of movement patterns associated to single-limb lameness, helping clinicians identify the lame limb. However, using such a model cannot help further differentiate towards the affected structure. Using patient data and collaborations on projects where lameness is expected due to other interventions may provide insights into natural causes of, and long-term adaptations to lameness. Moreover, the use of data from horses with naturally occurring lameness aids the transition to animal-free innovations for research.

Embrace biological variation

No matter which tools or settings are used to study locomotion, variability is inherent to data obtained from biological systems. Moreover, movement variability is an essential factor in the differentiation between healthy and pathological gait in humans.[13] In **chapter 5** it was shown that group-level adaptations could be revealed with both continuous as well as discrete methods to describe the data. There, we already argued that summarising curves of different strides within an animal can result in a loss of information, as peak angles may cancel each other out when the timing differs between strides. In addition, subtle individual adaptations to interventions might be lost when data is averaged over a group of horses, which advocates the evaluation of horses on an individual level (**chapter 5**).[14] This approach better represents all possible patterns one may encounter when observing horses outside of a research setting. In **chapter 6**, the choice was made to present the data per horse individually. As the sample population was small (three horses), it was of no use to present ranges or other point estimates of the data. Even in this small and homogeneous group of animals, studied under highly standardised conditions (i.e. treadmill locomotion), large inter- and intra-individual differences were found. Furthermore, in **chapter 7**, we advocate sharing all data supporting the study findings. Given the heterogeneity of the general population of horses, reporting only group means resulting from a study provides limited information that is of little use for other scientists, or clinicians and other professionals assessing individual animals. Therefore, group results should be supplemented by individual results to do justice to the often large variability in results from the investigated population.

Getting to a better understanding

In the previous paragraphs, we have mainly discussed equine locomotion in both healthy and pathological conditions. But much less is known about how horses actually move and why they change their locomotion pattern in response to pain in a typical way. Explaining gait patterns within the mechanics framework can provide a better understanding of these phenomena. Using simple mechanical models and Newton's second law of motion, $F = m*a$, many of the adaptations to lameness may be explained.

Can adaptations related to pain-induced lameness be explained by gait mechanics?

Clinical examination of lame horses is a time-consuming process. Finding out which limb is, or which limbs are lame, is only step one of a process in which several analgesic blocks can be necessary to determine which structure is affected. Part of this process's complexity is caused by the fact that the vast majority of deviations in a horse's gait pattern can be caused by various underlying motor impairments or even by different circumstances. For example, a trotting pattern characterised by an asymmetry in the vertical motion of the pelvis can be caused by acute hindlimb lameness due to pain in the hoof (**chapter 4**), but can also have its origin in other structures in the limb[15]. Moreover, gait pattern deviations are not always a direct result of a motor impairment but can represent a compensation strategy for a primary motor impairment. As such, a compensatory asymmetry in the head's vertical motion typically associated with forelimb lameness (**chapter 3**) can be caused by a primary hindlimb lameness (**chapter 4**)[16].

For bilateral lameness, understanding the mechanics of gait adaptations is even more valuable. The absence of asymmetries makes it extremely difficult to detect the lameness, both when using visual observations and when using systems measuring kinematics to quantify gait. In **chapter 5** we have shown that at trot, fetlock hyperextension decreases at impact after lameness induction and pelvis roll at midstance is increased. This is also represented by increased hip-hike stance asymmetry in unilateral hindlimb lameness (**chapter 4**). The principles of the spring-mass model can be used to explain these results. As such, it seems that the limb compresses less during the stance phase, decreasing the dorsoventral movement of the centre of mass and the limb's loading.

Muscles move the body

Ground reaction forces are results of locomotion but do not explain how the movements are produced. While the previous paragraphs focussed on the kinematics of locomotion, another aspect concerns the control of the muscles that generate the movements. During locomotion, the timing of muscle activations is critical to produce efficient and well-coordinated gait patterns. In its simplest form, a muscle contraction causes a moment around the joint resulting in movement. However, muscle activation serves many purposes during functional tasks such as locomotion. As such, muscles support the weight of the body against gravity, store elastic energy[17,18] and dampen impact vibrations[19]. It is then logical that any changes in the muscles themselves (including their tendons), their neural control and/or the joints will have an impact on the body system as a whole. The many degrees of freedom of an animal's body, though, makes the type and amount of this impact difficult to predict. In any case, the causes of the observed kinematic impairments may be better understood by analysing muscle function, i.e. how the movement is produced. Even though within this thesis we focused on how to measure muscle function instead of what the muscles actually do (**chapter 6**), we think that insight in their actions will aid the development of a reliable body of knowledge to further understand muscle function during equine locomotion.

Future perspectives

In a scientific context

Several suggestions for future research in the field of locomotion analysis in horses were already mentioned in the previous paragraphs. However, some additional recommendations for future research are highlighted below.

Vertical movement asymmetry is only a result of the adaptive changes in the locomotor pattern, not a goal in itself. To gain insight into the mechanics of lameness, there is a need for a thorough investigation into the application of mechanical models to equine locomotion in both sound as well as lame conditions. In hindsight, the data presented in this thesis would be well suited for this purpose. The development of comprehensive inverse dynamics models, integrating kinetics, kinematics and muscle function would be a great starting point. These models can be built using existing data and may also benefit the transition to animal-free innovations for research.

Technological developments increasingly provide opportunities to study animals in a more real-life setting. Sensors are becoming smaller and lighter or are even integrated in fabrics. This may simplify the data collection process, allowing for more data to be collected and several aspects of locomotion to be captured simultaneously. In addition, open science is becoming standard practice. All these aspects benefit the development of large comprehensive datasets. In time, these should be used to further understand the complexity of locomotion in an ecologically valid setting.

Complexity comes at the cost of interpretability**.** And yet, reductionism can be criticised for being so simple that it cannot accurately represent natural locomotor behaviours. Machine learning approaches to help interpret the data have proven to be promising to solve this issue. As such, in this thesis, they have been used for the automatic detection of footfalls, the direction of movement of the horse, the gait performed and the surface context. Furthermore, computer vision is a new and promising technique for kinematic analysis that has already been utilised to detect gait asymmetries[20] or even facial expressions[21]. These techniques pave the way towards understanding locomotor data with immense complexity, while still reducing the complexity of relevant data collection and processing. This is only the start of the possibilities that machine learning can bring in the near future. We believe machine learning approaches, applied to large datasets, are necessary to comprehend locomotion in all relevant environments and the impact of lameness.

Traditionally, both fundamental and applied research in the field of equine locomotion has been conducted by researchers with widely differing backgrounds. This diversity is important and should remain. Clinical applications are a large portion of the research output, which benefits equine welfare. Developing a comprehensive understanding of equine locomotion requires multidisciplinary cooperation and knowledge sharing between horse owners, engineers, clinicians and movement scientists. Therefore, future research in the field of animal locomotion should aim at a multidisciplinary approach.

In a societal context

There is an increasing societal pressure on the equine industry's 'licence to operate'. As such, a recent report stated that there is a need to break with the view of the horse as an athlete and recognise instead that the participation of horses in competitions is only about human interests and ambitions.[22] They do not find it problematic *per se* that horses are used for sport, but that their use entails a risk that the horses' welfare is compromised. It is emphasised that it is unacceptable for horses to suffer pain when performing sporting activities. For the latter, locomotion analysis might have an important role, as slowly, subjective reasoning in this ethical discussion is making way for objective arguments. The previously described technical developments may play an important role here. As we work towards a more comprehensive understanding of natural equine locomotion, and simultaneously make it easier to measure animals in their everyday environment, equine husbandry, including clinical practice can become increasingly evidence-based and eventually reach individual animal owners. This seems necessary, as 75% of horses in regular work are deemed lame when subjected to a subjective lameness exam[23] and injury rates in competing horses remain high, with 33% of dressage horses being reported lame in a single year[24]. When objective measures were applied to quantify asymmetries, a similarly large proportion (72.5%) of a group of 222 horses had significant movement asymmetries indicative of lameness.[25] Quantifying locomotion and understanding the stress and strain on a horse's body during exercise may aid to achieve better welfare in animals and prevent injuries leading to lameness.

Concluding thought

When it comes to clinical care, understanding normal function is a prerequisite to understanding locomotion in pathological conditions. Familiarity with kinetics, kinematics, and muscle function in sound animals provides a reliable body of knowledge aiding the diagnosis, prevention and rehabilitation of those who have impaired motor function. Awareness of and insight in these three components by themselves offers opportunities to understand locomotion further. Together, however, these cues for comprehensively capturing equine locomotion may enable a 'three-dimensional differentiation' between normal, pathological and compensatory gait patterns.

"The whole is something besides its individual parts."

- Aristotle

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Addendum

Nederlandse samenvatting
Kinderproefschrift
PhD portfolio
Curriculum vitae
Dankwoord

Nederlandse samenvatting

Het paard in beweging: signalen om de complexiteit te vatten

Locomotie is een fundamenteel onderdeel van het leven. De mogelijkheid om van de ene plaats naar de andere te bewegen stelt dieren in staat om met hun omgeving te interacteren. Dit maakt dat locomotie een essentiële factor is voor het laten zien van natuurlijk gedrag, gezondheid en het welzijn van een dier. Het is dan ook niet verrassend dat er al sinds het begin van de wetenschap interesse is in hoe dieren zich voortbewegen. De wetenschappelijke benadering voor het bestuderen van locomotie volgt over het algemeen de ontwikkeling van de wetenschap in het algemeen. Zo was in de oudheid de benadering voornamelijk observationeel en pas na de Renaissance, met toenemende middelen en technologieën, werden experimentele studies langzaam gangbaar. Locomotie is echter ongelooflijk complex en als gevolg daarvan is de wetenschappelijke benadering altijd sterk reductionistisch van aard geweest. De afgelopen decennia heeft er echter een exponentiële groei plaatsgevonden van met name relevante technologieën en rekenkracht. Dit maakt het mogelijk om naar een meer holistische benadering in de studie van locomotie toe te werken.

Dit proefschrift hoopt bij te dragen aan de ontwikkeling van een steeds meer holistische benadering voor het bestuderen van locomotie. Het onderwerp van deze studie is het paard, een van de zeldzame soorten die gedomesticeerd is vanwege zijn locomotorisch potentieel en, wat betreft dit potentieel, de meest bestudeerde niet-menselijke diersoort. Dit wordt gedaan door bestaande technieken toe te passen om verschillen tussen rassen te karakteriseren, minder gebruikelijke gangen te onderzoeken en te verkennen hoe pijn gerelateerd aan locomotie tot uiting komt in verschillende contexten. Daarnaast worden overwegingen gepresenteerd om de methodologische gereedschapskist in de disciplines van zowel kinematica als spierfunctie uit te breiden.

Korte samenvatting van ieder hoofdstuk

Hoofdstuk 2 beschrijft een observationele studie met 65 paarden. Het doel was om de verschillen tussen rassen en gangen in kaart te brengen wat betreft de verticale beweging van het bovenlichaam en temporale pas-parameters. Drie verschillende rassen (Warmbloed, Iberisch en IJslands) en vier gangen (stap en draf voor alle rassen, ook tölt en pace voor IJslanders) werden beoordeeld. De belangrijkste uitkomstparameters waren het verticale bewegingsbereik van het bovenlichaam (ROMz), timing van de verticale verplaatsing minima en maxima, en de duur van de pas, stand- en zwaaifase.

De resultaten toonden aan dat des te kleiner het ras, des te kleiner de ROMz van het bovenlichaam en hoe korter de duur van de pas, stand- en zwaaifase. Dit kan een logisch gevolg zijn van het hebben van kortere ledematen. De timing van verticale verplaatsing minima en maxima kon worden verklaard door de mechanica achter de gangen geclassificeerd als 'lopen' en 'rennen'. In stap, een gang geclassificeerd als lopen, waren de verticale verplaatsingsminima van de schoft en het bekken nauw verbonden met het moment waarop de twee contralaterale ledematen beide contact hadden met de grond; dus het moment waarop de twee voor- of achterbenen maximaal gespreid waren. De timing van de verticale verplaatsingsmaxima waren in de tijd gerelateerd aan het midden van de stand fase van de voor- en achterbenen. Voor rennen (draf, tölt en pace) waren de verticale verplaatsingsminima nauw verbonden in tijd aan het midden van de stand fase, terwijl de maxima werden bereikt rond de afduwfase.

Hoofdstuk 3 en 4 beschrijven twee experimentele studies waarin kreupelheid werd geïnduceerd in een voorbeen bij tien IJslandse paarden (**hoofdstuk 3**) en in een achterbeen bij elf IJslandse paarden (**hoofdstuk 4**). Het doel was om de veranderingen in kinematica (ruimtelijk en temporaal) die verband houden met deze geïnduceerde kreupelheid te beschrijven tijdens stap, draf en tölt. De data werden verzameld onder veldomstandigheden, zowel aan de hand (stap en draf) als tijdens het berijden van de paarden (stap, draf en tölt). Voornamelijk de asymmetrie in de beweging van het bovenlichaam en de timing tussen de benen werden beoordeeld.

Deze studies toonden aan dat 'traditionele' kreupelheidsparameters ook bij IJslandse paarden wijzen op kreupelheid aan voor- en achterbenen in stap en draf. In tölt konden deze parameters ook onderscheid maken tussen normale en kreupelheidsomstandigheden aan de achterbenen, maar niet goed voor kreupelheid aan de voorbenen. Bovendien bewegen IJslandse paarden überhaupt minder symmetrisch in tölt dan in stap en draf, zelfs zonder inductie van kreupelheid. Dit maakt het lastig om onderscheid te maken tussen gezonde en ongezonde toestanden op basis van asymmetrie van het bovenlichaam in tölt. Wat betreft de timing tussen de benen vermindert kreupelheid de relatieve tijd (als percentage van de pasduur) van de impact van het gezonde naar het contralaterale been bij alle gangen, maar niet andersom. De timing tussen de benen kan dus een waardevolle parameter zijn om onderscheid te maken tussen normale en pathologische condities in het paard.

Hoofdstuk 5 beschrijft een experimentele studie waarin verschillen werden beoordeeld tussen continue en discrete analysemethoden. Deze methoden werden toegepast op data van een studie over langdurige bilaterale kreupelheid aan de achterbenen van zestien Shetland pony's. Deze pony's namen deel aan een kraakbeenherstelstudie, waarbij ganganalyse werd gebruikt als klinische uitkomstmaat. Volledige lichaamskinematica werd vastgelegd met 3D-optische bewegingsregistratie terwijl de pony's draafden op een loopband.

Onze resultaten tonen aan dat continue data-analyse gunstig kan zijn voor de evaluatie van bilateraal kreupele pony's. Met name omdat deze bij bilaterale kreupelheid niet asymmetrisch worden wat betreft verticale beweging van het bovenlichaam. De twee methoden zijn complementair: discrete data geven gemakkelijk te interpreteren waarden, en continue data verschaffen aanvullende informatie over de timing en duur van de veranderingen. Bovendien kan continue data-analyse aanpassingen in de beweging onthullen die buiten piekwaarden plaatsvinden (welke vaak goed beschreven worden met discrete data). De belangrijkste veranderingen in beweging werden waargenomen in de bekkenrotaties (zowel pitch als roll), en in timing tussen de benen.

Hoofdstuk 6 beschrijft een observationele studie over het effect van elektrodelocatie op metingen van spieractiviteit van paarden met behulp van oppervlakte elektromyografie (EMG). Het doel was om aanbevelingen te doen over elektrodelocatie voor het verzamelen van oppervlakte EMG data bij paarden. Oppervlakte EMG data werden verzameld van twintig spieren van drie Warmbloed paarden die draafden op een loopband. De belangrijkste uitkomstparameter was signaal-ruisverhouding (SNR), en locaties werden vergeleken binnen een spier en paard.

Op basis van de resultaten van deze studie werden elektrodelocaties voorgesteld voor zestien van de twintig gemeten spieren bij Warmbloed paarden. Deze resultaten kunnen helpen bij het standaardiseren van methodologieën binnen en tussen onderzoeksgroepen. Wij geloven dat dit het vakgebied ten goede kan komen en kan helpen bij het creëren van een groot en betrouwbaar fundament van bewijsmateriaal over spieractivatie bij bewegende paarden. Het werk ten behoeve van de standaardisering is nog niet voltooid, en de aanbevelingen kunnen veranderen met opkomende nieuwe technologieën.

In **Hoofdstuk 7** worden de datasets beschreven die zijn gebruikt voor hoofdstukken 3, 4, 5 en 6 van dit proefschrift (dat wil zeggen de datasets van de studies naar inductie van kreupelheid aan voor- en achterbenen, de bilaterale kreupelheidsstudie en de dataset gebruikt voor het bepalen van elektrodelocatie voor oppervlakte-EMG-metingen). Met dit hoofdstuk streven we ernaar open wetenschap te bevorderen door de gegevens die onze bevindingen ondersteunen te delen.

Conclusie

Als het gaat om klinische zorg voor dieren, dan is het begrijpen van normale functie een voorwaarde om locomotie in pathologische omstandigheden te begrijpen. Kennis over kinetica, kinematica en spierfunctie van gezonde dieren biedt een betrouwbaar fundament dat helpt bij de diagnose, preventie en revalidatie van degenen met verminderde motorische functie. Bewustzijn van, en inzicht in deze drie componenten op zichzelf biedt kansen om locomotie verder te begrijpen. Samen kunnen deze aanwijzingen voor het vastleggen van de complexiteit van locomotie een 'driedimensionale differentiatie' mogelijk maken tussen normale, pathologische en compenserende looppatronen.

"Het geheel is meer dan de som der delen"

~Aristoteles

Kinderproefschrift

Als een paard pijn aan een been heeft wanneer hij beweegt, dan noemen we hem kreupel. Dat kreupel zijn wil je snel herkennen, zodat je er zo snel mogelijk iets aan kunt doen. Maar hoe herken je pijn bij paarden eigenlijk? Ik ben Ineke, paardenonderzoeker, en ik leg je uit hoe dat zit.

(A)symmetrisch

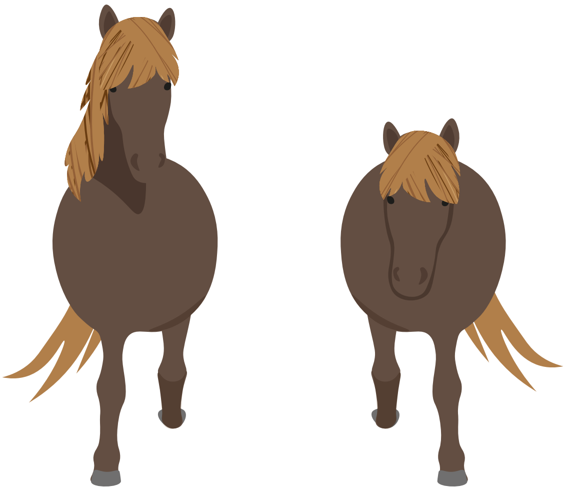
Normaal, als je nergens last van hebt en je loopt of rent op een rechte lijn, dan beweegt de linker kant van je lichaam hetzelfde als de rechter kant van je lichaam. Dat noemen we dan “symmetrisch” bewegen. Zo verspil je zo min mogelijk energie en belast je beide kanten van je lichaam hetzelfde. Daarom doen eigenlijk alle dieren dat, dus ook mensen en paarden.

Maar als jij ergens last van hebt, dan ga je anders bewegen om zo min mogelijk pijn te voelen. Als je bijvoorbeeld door je enkel bent gegaan, dan houdt je het been dat zeer doet stijver, waardoor je heup ook hoger blijft, dan het been dat niet zeer doet. Ook val je sneller op de voet waar je geen pijn aan hebt. Een beetje zoals een piraat met een houten been loopt. Dit doe je allemaal om pijn zo min mogelijk te voelen als je loopt. Paarden doen eigenlijk precies hetzelfde. Dit “anders” bewegen noemen we in het onderzoek asymmetrisch bewegen, dus niet meer hetzelfde aan de linkerkant als aan de rechterkant.

Het gekke is, als een paard pijn heeft aan een been, dan zie je niets raars aan dat been zelf,. Toch kun je de pijn herkennen, en zelfs verschil zien tussen ppijn aan een voorbeen of aan een achterbeen.

Ja-knikken of heupwiegen

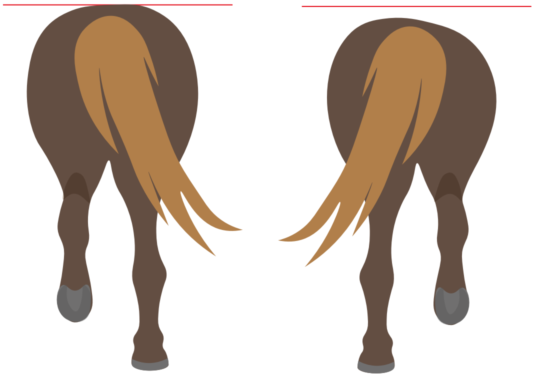
Als een voorbeen zeer doet, dan gaat hij namelijk ‘ja-knikken’ met zijn hoofd, wat je het beste kan zien als hij naar je toe draaft. Als hij het been dat niet zeer doet op de grond zet, dan beweegt hij zijn hoofd verder naar beneden dan wanneer hij zijn been op de grond zet dat wel zeer doet. Terwijl normaal, als een paard geen pijn heeft, zijn hoofd bijna niet op en neer beweegt in draf.



Dit paard heeft pijn aan het rechter voorbeen. Het hoofd is hoger wanneer het pijnlijke been aan de grond is dan wanneer het gezonde been aan de grond is.

Maar als het achterbeen van het paard zeer doet is dit moeilijker te zien. Hij zal dan het midden van zijn heupen, boven de staart, verder naar beneden bewegen wanneer het been dat niet zeer doet op de grond staat dan wanneer het been dat wel zeer doet op de grond staat. Wat hij ook nog kan doen is meer met zijn heupen draaien aan de kant van het been dat zeer doet. Eigenlijk een soort heupwiegen alsof je op hakken loopt, maar dan aan één kant.

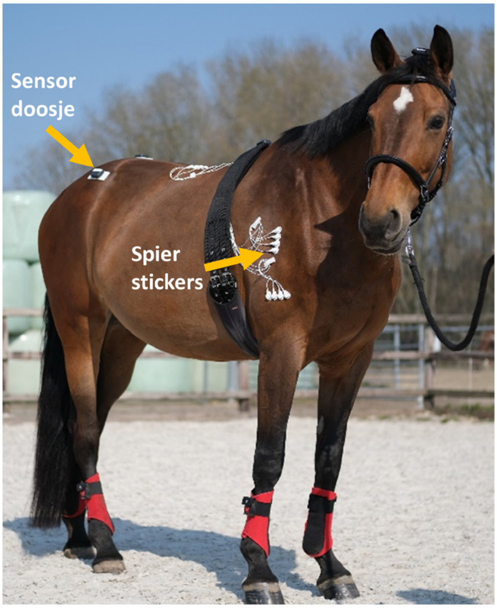
Dit ja-knikken en heupwiegen zijn voorbeelden van het 'asymmetrisch bewegen', en hoe meer pijn het paard heeft, hoe meer asymmetrisch het paard zal bewegen.



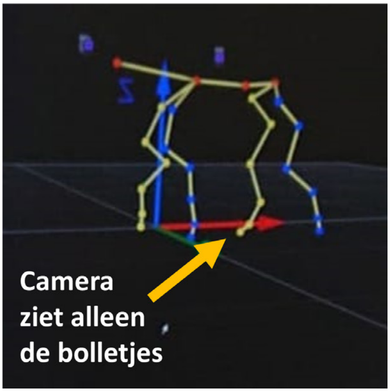
Dit paard paard heeft pijn aan het rechter achterbeen. De heup is hoger wanneer het pijnlijke been aan de grond is dan wanneer het gezonde been aan de grond is.

Beweging meten

Vaak is het lastig om het ja-knikken of heupwiegen, de asymmetrie, met je blote ogen te zien. Om dit op te lossen kunnen dierenartsen of onderzoekers zoals ik de beweging van een paard meten. Je kan op verschillende manieren de beweging meten, bijvoorbeeld door de beweging zelf te meten (hoe ver beweegt een been heen en weer?), de spieren te meten (wanneer is een spier aan of uit?), of door te meten met hoe veel kracht het dier op de grond komt bij elke pas. Ik heb deze manieren zelf gedaan door kleine doosjes met sensoren er in, bolletjes die door speciale cameras gezien worden, en door een soort stickers op de spieren van een paard te plakken.



Het is handig om de beweging van een paard te meten als de pijn (nog) niet heel erg is, zodat het paard snel geholpen kan worden en de pijn niet erger wordt. Bij weinig pijn is de asymmetrie soms maar een paar millimeter, wat erg lastig te zien is. Het meten van de beweging kan ook handig zijn als het paard niet draaft maar bijvoorbeeld tölt, wat heel snel gaat, of bij kleinere paarden, zoals bijvoorbeeld shetlanders of IJslanders. Kleinere paarden maken meestal kortere pasjes en daardoor bewegen ze sneller en is de beweging zelf ook kleiner. Hoe sneller en kleiner iets beweegt, hoe moeilijker het is om een asymmetrie met je ogen te zien. Denk maar eens aan hoe moeilijk het te zien is of bijvoorbeeld een vlieg beide vleugels beweegt als hij voor je vliegt.



Nog lastiger om te herkennen is als het paard aan twee benen tegelijk pijn heeft. Want als je aan twee benen ongeveer evenveel pijn hebt dan helpt asymmetrisch lopen niet om je pijn te verminderen. Hoe een paard precies loopt als hij aan twee voorbenen tegelijk kreupel is, dat is nog niet bekend. Maar wat we wel weten is dat als een paard kreupel is aan twee achterbenen, dat hij dan minder buigt in zijn rug en meer draait met zijn heupen, vooral vlak voordat hij afzet met een achterbeen.

Paarden en mensen

Paarden zijn gemaakt om te bewegen. In het wild moeten ze rondlopen om eten en drinken te vinden of te vluchten om te overleven. Maar ook in hier & nu; paarden en mensen trekken al lang samen op. Mensen gebruikten paarden vroeger als werkdier, bijvoorbeeld om akkers te bewerken of om mensen in een koets van a naar b te brengen. Nu hebben we hier machines voor en gebruiken we paarden vooral voor sport en vermaak. Dit maakt het extra belangrijk dat het paard geen pijn heeft wanneer die beweegt. Wanneer jouw paard kreupel is, dan is het dus heel belangrijk om daar snel iets aan te doen om te voorkomen dat het erger wordt. Door onderzoek te doen bij paarden begrijpen wij steeds beter hoe een paard beweegt bij pijn. Hierdoor kunnen we paarden sneller beter maken wanneer ze last hebben bij het bewegen. Daarom vind ik mijn werk zo leuk. Ik help mee om paarden weer lekker te laten bewegen!

Test je kennis!

Opdracht: Maak een flipboekje en zoek uit welk been zeer doet.

Een flipboekje is eigenlijk een hele korte tekenfilm. Op ieder vel van het boekje staat een afbeelding die steeds een heel klein beetje verandert. Als je het hele boekje heel snel achter elkaar doorbladert ('flipt') zie je de pony bewegen en heb je een klein animatiefilmpje gemaakt!

Kan jij zien waar de pony pijn heeft? Het antwoord kan je vinden op pagina 157.

Wat heb je nodig?

1. De volgende pagina's van dit boekje
2. Een schaar
3. Een nietmachine, lijm of naald en draad

Stappen

1. Knip de pagina's hierna over de stippellijn in stukken.
2. Leg ze op volgorde (1 bovenop en 24 onderaan) en niet, plak of stik ze aan de hele linker kant aan elkaar. Zorg ervoor dat alle vellen netjes op elkaar liggen voordat je dit doet, zodat de pony steeds op dezelfde plek staat.
3. Nu is het boekje klaar voor gebruik! Als je het boekje flipt zal er een miniflimpje ontstaan.

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De pony heeft pijn aan het rechter voorbeen

PhD portfolio

Courses and workshops	Year
Laboratory Animal Science	2019
Species specific Laboratory Animal Science – Horses and Donkeys	
Writing a scientific paper	2020
Analytic storytelling	2020
Research planning and time management	2020
Responsible conduct of research: how to do it right?	2020
Scientific artwork – data visualization and infographics	2021
Psychological flexibility	2021
Achieving your goals and performing more successfully in your PhD	2021
Supervising research of master students	2022
Digital tools and objective methods for motion research in animals	2022
Science communication: relevant and clear	2023
Facultaire leergang basiskwalificatie Onderwijs	2022

Other	Year
Breaking science pitching competition	2023
Vet PhD Council: represent PhDs	2020 - 2023
Supervision of bachelor and master research projects	2019 - 2023
Bachelor: Maud van der Heiden; Marit Dijkstra; Laura de Rek	
Master: Ann-Sofie Scheike; Emma Spaan; Alisa Maassen	
Conference organization	2023
International conference of canine and equine locomotion, Utrecht	

(Inter)national conferences and presentations	Year
Veterinary science day, Bunnik	2019
Veterinary science day, Bunnik	2022
Short oral presentation	
Veterinary science day, Bunnik	2023
International conference of equine exercise physiology, Uppsala	2022
Poster and oral presentation, best poster award	
International conference of canine and equine locomotion, Utrecht	2023
Workshop , poster and oral presentation	
Locomotion in practice, Utrecht	2023
Oral presentation	

Curriculum vitae

Werkervaring

Promovenda

Utrecht Universiteit| mei 2019 – oktober 2023
Promotieonderzoek naar locomotie van het paard. Enerzijds wordt onderzocht hoe het gezonde paard beweegt en hoe we dat kunnen meten. Anderzijds werd er gekeken naar hoe de beweging veranderd als er pijn tijdens beweging is.

Nevenactiviteiten

Representatie van veterinaire PhDs

Utrecht Universiteit | 2020 –2023
Eerst als lid en later als voorzitter van de veterinaire PhD council, welke als doel heeft de belangen van de vet PhDs te behartigen en PhDs te verenigen.

Verzorgen van onderwijs

Utrecht Universiteit | 2021 – heden
Verzorging en ontwerp van onderwijs rondom het thema locomotie voor masterstudenten diergeneeskunde. Enerzijds over kreupelheidsdiagnostiek, anderzijds een cursus over 'animal movement science'.

Opleiding en nascholing

Leergang basiskwalificatie onderwijs

Utrecht Universiteit | 2022 – heden

PhD opleiding Graduate school of life sciences (GS-LS)

Utrecht Universiteit, Universitair medisch centrum | 2019 – 2023

Master human movement sciences

Vrije Universiteit | 2017 – 2019
Met een specialisatie in revalidatie en een focus op de biomechanica van het bewegen.
Nevenactiviteit: Team manager PULSE racing: fietsen voor mensen met een dwarslaesie m.b.v electrostimulatie

Bachelor fysiotherapie

Tim van der Laan | 2016 – 2018 (niet afgemaakt)
Deeltijd opleiding fysiotherapie, waarbij ik mij wilde focussen op de implementatie van wetenschap in de fysiotherapie.

Bachelor Bewegingswetenschappen

Rijksuniversiteit Groningen | 2013 – 2016
Met een focus op neurorevalidatie en een minor neuroscience (vrije universiteit)
Nevenactiviteit: commissielid IFMSA en student-assistent bij het international office

Atheneum

H.N. Werkman college | 2006 – 2012
Combinatieprofiel natuur & gezondheid en natuur & techniek, keuzevak muziek

Dankwoord

Het is klaar. C'est ça.

Wat heb ik uitgekeken naar dit moment! Enerzijds vanwege de blijdschap, opluchting en dankbaarheid die ik ervaar voor deze mijlpaal. Anderzijds vanwege de behoefte om mijn ingehouden adem op weg naar deze 'finish' met een diepe zucht eindelijk los te kunnen laten. Dat moment is nu eindelijk gekomen.

Zoals bij de meeste belangrijke mijlpalen in iemands leven was de uitvoering van mijn promotieonderzoek en het schrijven van dit proefschrift absoluut onmogelijk geweest zonder de hulp en steun van mensen in mijn directe omgeving. Daarom wil ik alle lieve/slimme/grappige/zorgzame/eerlijke mensen die mij de afgelopen jaren op enige wijze van raad of daad hebben voorzien ontzettend bedanken. Mijn begeleiders, collega's, de proefpaarden, leden van de leescommissie, vrienden, familie en Nander; de woorden die vatten wat ik zeggen wil zijn moeilijk te vinden. Die komen persoonlijk misschien beter tot hun recht.

Acknowledgements

It is done. Det er det.

How I have been looking forward to this moment! On the one hand, because of the joy, relief and gratitude I experience for this milestone. On the other hand, because of the need to finally let go of my long held breath on the way to this ‘finish’ with a deep sigh. That moment has finally arrived.

As with most important milestones in one’s life, conducting my PhD research and writing this dissertation would have been absolutely impossible without the help and support of people close to me. That is why I would like to thank all the sweet/smart/funny/caring/honest people who have provided me with advice or assistance in any way over the past few years. My supervisors, colleagues, the test horses, members of the reading committee, friends, family and Nander; the words that capture what I want to say are difficult to find. Perhaps it would do them more justice to say them in person.