Journal Pre-proof

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PII: S0737-0806(22)00214-3
DOI: https://doi.org/10.1016/j.jevs.2022.104077
Reference: YJEVS 104077

To appear in: *Journal of Equine Veterinary Science*

Received date: 24 December 2021
Revised date: 6 July 2022
Accepted date: 6 July 2022

Please cite this article as: Milena D. Scheidegger , Vinzenz Gerber , Gaudenz Dolf , Dominik Burger , Shannon Axiak Flammer , Alessandra Ramseyer , Quantitative gait analysis before and after a cross-country test in a population of elite eventing horses, *Journal of Equine Veterinary Science* (2022), doi: https://doi.org/10.1016/j.jevs.2022.104077

Original Article | Original Research

Quantitative gait analysis before and after a cross-country test in a population of elite eventing horses

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Highlights

- High occurrence of gait asymmetries in elite eventing horses deemed fit-to-compete
- Gait asymmetries are not worsened after strenuous exercise
- Shortened stride duration the day following the cross-country course

Abstract

Early recognition of lameness is crucial for injury prevention. Quantitative gait analysis systems can detect low-grade asymmetries better than the human eye and may be useful in early lameness recognition. The aims of this study were 1) to investigate the frequency of gait asymmetries based on head and pelvic movement in elite eventing horses using inertial mounted measurement units and 2) to assess the association between asymmetries and muscle enzymes and blood lactate (LA) levels post exercise. Movement asymmetry of the head, wither, and pelvis were quantified in 33 elite eventing horses prior to and one day after the cross-country test of three Concours Complet International (CCI3* and CCI4*) events held three weeks apart. The effects of LA concentration immediately after completion of the cross-country course and of serum levels of creatine kinase (CK) and aspartate amino-transferase (AST) four hours post-exercise on gait asymmetry parameters were tested with linear models. A total of 58% and 77% of the 33 horses exhibited gait asymmetries that exceeded published threshold values before and after the cross-country course, respectively. The magnitude of pre-existing gait asymmetries was not significantly increased after the cross-country test and no associations with post-exercise levels of CK, AST, or LA were detected. The stride duration was significantly shorter the day following the cross-country test and was associated with LA, the age and the weight of the horses. In conclusion, a majority of the horses studied presented gait asymmetries and strenuous exercise resulted in decreased stride duration but did not worsen gait asymmetries.

Keywords: *Equine, gait asymmetries, lameness, muscle enzymes; orthopedic*

1. Introduction

Lameness is a major performance-limiting factor in sport horses and has substantial economic and emotional consequences. Therefore, early detection of lameness is fundamental for prevention of injuries and the improvement of animal welfare in equestrian sports. Standard practice for the identification of gait asymmetries is a full lameness examination with visual gait assessment performed by an equine veterinarian. However, numerous studies have highlighted the limitations of subjective gait analysis, including its low sensitivity to detect mild lameness. Furthermore, subjective gait analysis varies with observer experience, has a poor inter-rater agreement and is influenced by expectation bias [1, 2, 3].

Quantitative gait analysis systems based on inertial mounted measurement units (IMUs) are a modern and practical tool that were developed for non-biased objective gait assessment in horses. They are usable in the field and have been shown to be superior to subjective lameness evaluation for detection of mild lameness and low-grade asymmetries [4, 5]. Since the development of these technologies, an increasing number of studies have demonstrated that up to 50-72% of ridden horses considered sound by their owners showed significant head and pelvic motion asymmetries when assessed objectively [6, 7, 8, 9]. Consequently, the ability of the owners and riders to detect lameness is questionable and this might represent a problem for animal welfare [7]. However, these results must be interpreted with

caution. Asymmetric gait patterns can be related to a pain-related lameness, but may also be due to non-painful conditions such as mechanical abnormalities or anatomical variations [10, 11].

Eventing is a highly challenging equestrian sport where horses are tested in dressage, cross-country and show jumping over one to three days. The cross-country test is the most physically demanding for the horses. Injuries to the musculoskeletal system are among the most frequent reasons for wastage and withdrawal of high level eventing horses from competition [12]. Therefore, early recognition of lameness is a corner stone in injury prevention.

During strenuous exercise, increased levels of the muscles enzymes creatine kinase (CK) and aspartate aminotransferase (AST) are commonly observed in healthy horses. The serum levels of these enzymes are widely used as indicators of muscle damage [13, 14]. Exercise-related elevation of blood lactate (LA) is also used to assess fitness and training methods in sport horses [15]. Increases in serum levels of CK, AST and LA are proportional to exercise duration and intensity, might indicate muscle fatigue and may be related to development of delayed onset of muscle soreness (DOMS), which can have an influence on the gait symmetry pattern detected post exercise [16].

To our knowledge, no study has investigated the frequency of objectively measured gait asymmetries in elite eventing horses, nor the effect of intense exercise during the cross-country test on head and pelvic movement. Based on published data in populations of owner-assessed sound riding horses, we hypothesized that the frequency of gait asymmetries in eventing horses would be more than 50%. The objectives of the present study were (1) to quantify the frequency of gait asymmetries based on head and pelvic movement in a population of elite eventing horses, and (2) to investigate differences of magnitude in gait symmetry parameters measured before and after the cross-country test of a three-day-event and their association with levels of CK, AST and LA.

2. Materials and methods

2.1. Study design

A nonrandom cross-sectional study was performed under field conditions by the Swiss Institute of Equine Medicine (ISME) at the Institut Equestre National Avenches (IENA) in Avenches, Switzerland. Data were collected during three national three and four star Concours Complet International (CCI3* and CCI4*) events held three weeks apart in July and August 2020. These events were trainings organized in competition format according to the regulations of the Fédération Equestre Internationale (FEI) specifically for the Swiss National Eventing Team who were unable to participate in international competitions because of COVID-19 related travel restrictions. In comparison to official competitions, several adaptations were made that included shortening the cross-country test and adding a second show jumping test between the dressage and the cross-country test. Informed consent

and approval were obtained from the owners and riders of the horses, the eventing committee of the Swiss Equestrian Federation, and the Animal Experimentation Committee of the Canton of Vaud (permit number VD3585).

The events began on Friday mornings with the dressage test, followed by the first show jumping test on Friday afternoons. The cross-country phase took place on Saturdays on a 2850 m course with 26 efforts at a speed of 570 m/min (CCI4*) or a 2750 m course with 24 efforts at a speed of 550 m/min (CCI3*). Cross-country obstacles were built according to FEI guidelines. The competitive trainings ended on Sundays with a 2nd show jumping test.

2.2. Population

A total of 19 CCI3* and 15 CCI4* horses were invited to participate in the events. Horses were recruited based on convenience sampling. To be included, horses needed to be considered fit to compete as judged by their riders and by the team veterinarian. Exclusion criteria were visual evidence of lameness, or any clinical abnormalities detected during the first veterinary examination at arrival on site. The owners and riders of the horses could decide whether to participate in one, two, or three events.

2.3. Data collection

At each event, horses underwent a first veterinary examination at arrival on site, which included a general clinical physical exam, body weight (Platform scale PS3000, Brecknell LLC, Fairmont, MN, USA) and blood withdrawal from the jugular vein for routine hematology (Advia 2120i, Siemens Healthineers AG, Erlangen, Germany) and chemistry (Cobas c501, Roche Diagnostics International AG, Rotkreuz, Switzerland), profile.

Quantitative gait assessments were performed twice: during the first veterinary examination at arrival (Thursday night or Friday morning) and the day following the cross-country, before the final show jumping test (Sunday morning). In addition, jugular blood samples were withdrawn immediately after completion of the cross-country course for direct measurement of LA concentration using a portable hand-held device (Lactate Pro2, Axon Lab AG, Dättwil, Switzerland). Four hours after completion of the cross-country course, a final blood sample was taken to measure serum levels of CK and AST (Cobas c501, Roche Diagnostics International AG, Rotkreuz, Switzerland).

Quantitative gait assessments were performed with a commercial IMU-based gait analysis system (Equigait Ltd, Cheshunt, Herts, UK) that had been previously validated [17, 18]. Each horse was equipped with five IMUs (MTw2, Xsens, Enschede, The Netherlands) attached over the poll, to the withers, between both tuber sacrale and to the right and left tuber coxae. All horses were trotted in hand by their regular handler (groom/rider/owner) in

a straight line on a concrete flat surface of approximately 50 meters. Handlers were asked to trot at the horse's preferred speed, and not to interfere with the lead rope nor to restrict the head movements of the horses. Each horse was trotted a minimum of four lengths until a goal of 25 strides of steady-state trot were obtained. Data were collected simultaneously from the five IMUs and were transmitted wirelessly at a 100 Hz sample rate per channel, via Bluetooth, to a nearby laptop.

2.4. Data processing

Collected continuous data were segmented into stride cycles and further processed by the commercial software package of the system using custom-written MATLAB scripts (The Mathworks, Natick, MA, USA). For each stride, validated symmetry parameters were derived based on vertical displacement [19, 20] and mean values across strides and the average stride times (AvStrT) were calculated. The three symmetry parameters with a known correlation with lameness were selected for data analyses: the difference between the lowest position of the head (HDmin), withers (WDmin) and pelvis (PDmin) during the mid-stance phase of the left and right limb [20]. Sign convention for side of the gait asymmetries with negative values for left-sided asymmetries and positive values for right-sided asymmetries was used [20]. The frequency of gait asymmetries was calculated by applying the screening threshold for lameness developed by Pfau et al. [21], which is set at 14.5mm for HDmin (68.8% sensitivity and 88.9% specificity) and 7.5mm for PDmin (90% sensitivity and 93.3% specificity). This threshold has been calculated for similar MTx-based

IMU systems, with the aim to identify gait asymmetries exceeding the expected normal variation in a cohort of horses where the prevalence of lameness is unknown [21].

2.5. Statistical analysis

Statistical analyses were carried out using Stata/SE 16.1 (StataCorp LLC, College Station, Texas, USA). Descriptive analyses were performed for the target traits HDmin, WDmin, PDmin, AvStrT and for the independent variables CK, AST, LA, age and weight. Normality was tested with a Shapiro-Wilk W test. As a preliminary analysis, differences in the means of the target traits and independent variables with respect to the timing PRE-POST were tested with a two-sample *t* test. Effect of the independent variables on the target trait HDmin, WDmin, PDmin, AvStrT were tested with linear models and regression coefficients were calculated with an ordinary least square regression (OLS). The full OLS regression model for all target traits included the following fixed effects: sex, age, weight, event, timing PRE-POST, CK, AST and LA. Because a linear relationship between the continuous independent variables and the target traits could not be assumed, the fixed effects were entered as linear and quadratic terms where appropriate. The proportion of variance of the dependent variable that can be predicted by the independent variables in the model was estimated with the r^2 value. Because some horses participated in one, two, or three events, most horses had more than one observation, so we had to consider the possibility that repeated observations may not be independent of each others. To address this problem, a robust estimator of the error variances with the identity of the horses as cluster variable was used. The threshold for significance was set at $P < 0.05$.

3. Results

3.1. Population

Of the 34 horses that were invited to participate in the study, one was excluded because of abnormalities in the blood chemistry profile. A total of 33 horses (13 mares and 20 geldings) ranging in age from 7 to 18 years (median 12), weighing [mean ± SD (range)] 536 ± 38 (451− 631) kg were included. Represented breeds included Warmblood (*n*=34) and Thoroughbred (*n*=1) horses, regularly competing at CCI3* (*n*=18) and CCI4* (*n*=15) levels. All horses were in full training and were regularly checked by the team and their private veterinarians throughout the competition season. All horses were transported to the competition venue the day before or on the first morning of the event, except for three horses that were stabled on site. During the events, horses were housed in the same barn in individual box stalls and were fed their own individual regular diet. Seven horses participated in one event, nine horses participated in two events, and 17 horses participated in all three events.

3.2. Quantitative gait analysis

At each of the three events, horses were tested for gait asymmetry twice resulting in a maximum of six observations per horse. A total of 150 observations for 33 horses could be collected during the three events, but only 103 observations fulfilled the minimum requirement of 25 strides (Equigait manual, page 51). The comparison of the distributions of the target traits HDmin, WDmin, PDmin, and AvStrT having 25 or more strides with the corresponding distributions having 15 to 24 strides showed that all 145 observations with 15 or more strides could be used in the analyses [22]. One observation was excluded because the horse sustained a traumatic injury during the first show jumping test. Due to technical errors, another four observations were excluded. A total of 140 observations for 33 horses remained in the analyses. Seventy-four observations were recorded before the cross-country in 33 horses and 66 were recorded after the cross-country test in 30 horses; 128 observations formed 64 pairs with respect to the timing PRE-POST. The distribution of the 140 observations is shown in Table 1.

3.3. Descriptive statistics

The symmetry parameters were calculated from a total of 3980 strides with an average stride duration of 683 ± 37 ms. The average number of strides collected per observation was 28 ± 7(range 15 − 53) strides. Descriptive statistics for the dependent and the quantitative independent variables are presented in Table 2 and Table 3, respectively. The Shapiro-Wilk W test showed that HDmin, PDmin and AvStrT were normally distributed, but WDmin was not. However, after removal of one observation with an extreme value, normal distribution of WDmin could not be rejected anymore.

3.4. Frequency of gait asymmetries

Fourty-six percent of the total observations (65 of 140) were classified as outside the threshold values based on HDmin and/or PDmin at least once in 25 of 33 horses. Before the cross-country test, 35% (26 of 74) of the observations measured in 58% (19 of 33) of horses exceeded the threshold. After the cross-country test, 59% (39 of 66) of the observations measured in 77% (23 of 30) of horses exceeded the threshold. In the 128 paired PRE-POST observations, *t* tests showed significant difference in the means with respect to PRE-POST for AvStrT, which was 23.1 ms shorter after the cross-country (P = 0.000), and for CK, which increased of 61.66 U/L after the cross-country (P = 0.000). There was no significant difference in the means with respect to PRE-POST for HDmin, PDmin, and WDmin. Details of the *t* tests are provided in Table 4. Distribution of the observations that were above or below the lameness screening threshold are shown in Table 1 and comparisons of the target traits before and after cross-country are illustrated in Fig. 1.

3.5. Regression analysis

Results of the regression analyses are given in Table 5 and Table 6. For HDmin, the final model showed a small negative effect of body weight (F = -0.10, P = 0.039) and a positive effect of age (F = 0.30, P = 0.018), explaining 16% of the variance (r^2 = 0.16). AST had a

negligible negative effect on WDmin, explaining only 3% of the variance (F = -0.02, *P* = 0.041, r^2 = 0.03). No independent variable statistically influenced PDmin. The greatest effect was identified in the AvStrT variable which was negatively influenced by the age (F = -5.78, *P* = 0.015) and LA (F = -0.91, *P* = 0.003), and positively related to the weight (F = 0.31, *P* = 0.006) and the event (F = 11.51, *P* = 0.002), overall explaining 35 % of the variance (r^2 = 0.35)

4. Discussion

This study investigated head and pelvic movement asymmetry in a population of 33 elite eventing horses before and after the cross-country test of three consecutive three-day Concours Complet International competitive trainings. In agreement with our hypothesis and with previously published data in populations of owner-determined sound riding horses [6, 7, 8, 9], the present study detected a high frequency of objectively assessed gait asymmetries before (58% of horses) and after (77% of horses) the cross-country test. However, there was no statistical difference in the means of HDmin, PDmin and WDmin before and after the cross-country test in the 128 paired PRE-POST observations. Post-exercise levels of CK and LA showed no association with the gait asymmetries and post-exercise levels of AST had minor effects explaining only 3 % of the variance observed in WDmin. The stride duration was significantly shorter after the cross-country test and was associated with LA, the age and weight of the horses.

Despite the high frequency of gait asymmetries, all horses were deemed "fit-to-compete" by the veterinary staff during the two examinations, except for one horse that was withdrawn because of injury during the show jumping in the 2nd event. Since the development of quantitative gait analysis systems, an increasing number of studies have demonstrated that up to 50-72% of horses deemed sound by their owners were actually quantified outside normal values for gait asymmetries [6, 7, 8, 9]. However, the clinical relevance of these asymmetries remains unknown. Asymmetries are caused by variations in loading and force production between limbs [23] and do not necessarily indicate pain-related lameness. Mechanical and conformational abnormalities can also induce asymmetric gait patterns [10, 11]. If it is assumed that the gait asymmetries are pain-related, then an exacerbation might be expected after completion of a highly intense exercise such as the cross-country test, while conformational asymmetries resulting from biological variation or mechanical restrictions would be expected to remain unchanged. However, a full clinical lameness examination would have been necessary to exclude pain-related lameness.

Eventing, especially the cross-country test, carries a potential risk of traumatic injuries for horses and riders. Wastage and withdrawal from competition of eventing horses due to musculoskeletal injuries was reported to range from 28% in lower class eventing horses [24] to 45% in elite eventing horses [12]. Therefore, in the context of injury prevention, the evaluation of a horse's fitness to compete is mandatory and regulated by the FEI code of conduct for the welfare of the horses [25]. If one postulates that asymmetric horses have a higher risk of injury during intense exercise, then objective gait analysis system could be a

helpful tool for veterinarians to prevent traumatic injuries during competition by identifying asymmetric horses. However, the risk of overestimating lameness in competition horses is not negligible. One study quantified gait irregularities with a IMUs-based system during endurance races, and found significant disagreement between the objective measurements, which detected gait irregularities in 21 out of 22 horses, and the veterinary subjective evaluation [26]. During the three events of the present study, one horse sustained a traumatic injury during the first show jumping test. This horse showed no gait asymmetry before exercise that could have predicted a potential trauma. Nevertheless, gait asymmetries and low-grade lameness may have an impact on the quality of performance of competition horses. Further studies should address the relationship between gait asymmetries and performance in competitions.

The most marked effect detected by the statistical analysis was that the average stride time was significantly shorter after the cross-country test compared to before. Stride duration was also influenced by LA, the age and weight of the horses. Heavier horses showed longer strides, which might be because they are often taller horses. Horses with higher post-exercise LA concentrations and older horses exhibited shorter strides after the cross-country test. This observation was not related to gait asymmetries parameters and might be an effect of fatigue. Parkes et al. [27] showed that stride duration decreased with training and after a period off work in young racing Thoroughbreds. Furthermore, Munoz et al. [28] showed that endurance horses disqualified because of lameness had higher CK activity, which was also associated with a lower trotting velocity at the vet-gate. However, an association between muscles enzymes and stride duration could not be confirmed in the present study.

In the human athlete, DOMS is a common consequence of strenuous exercise that provokes tenderness and movement-induced pain 24-72 hours post exercise and is associated with elevated CK activity [16, 29]. DOMS has not been described in horses, but commonly observed elevations of serum muscle enzymes post exercise in healthy horses indicate a similar process, which could influence gait symmetry pattern. One study identified an association between blood LA concentrations measured immediately after show jumping competition and muscle soreness detected two days later in 10 horses [30]. In the current study, post exercise levels of CK, AST and LA were investigated as an indicator of muscular and metabolic fatigue [15, 31, 32, 33]. We found only a small and significant increase in CK activity four hours after the cross-country test but no effect on the gait symmetry parameters nor on stride duration measured the day following the cross-country test. There was no increased AST activity after the cross-county test and the final model detected a negligible effect of AST on withers symmetry in movement. However, the serum muscle enzymes levels were measured four hours after the cross-country test and AST peak activities may not have been captured. Blood LA levels measured immediately after the cross-country test were in the range of those reported by Kirsch et al. [32] in CCI3* and CCI4* eventing horses and had a small negative effect on AvstrT. Further studies are required to assess the effect of exercise and fatigue on stride duration.

4.1. Study limitations

This study was performed under field conditions and convenience sampling was used to recruit horses. It was not randomized and not blinded and therefore may be biased. Furthermore, the evolution of the gait asymmetries over the three different cross-country events were not further analyzed since potential treatments and management changes between the events could not be completely controlled. Indeed, without information on whether asymmetric horses received medical treatments or corrective shoeing, it was not possible to objectively evaluate if gait asymmetries improved or worsened over the nine weeks that the study lasted. Technical bias for IMUs measurements may be present. Indeed, even though inertial sensor systems are reported to outperform the human eye for detection of mild lameness [4], they are not without flaws. The IMUs must be very accurately placed on specific anatomical locations and a small difference in marker placement can create artificial asymmetries in the measured outcome [34]. Similarly, any abrupt or exaggerated movement of the horse or interaction between the handler and the lead line could influence the results. Furthermore, IMUs are not capable of identifying bilateral lameness on a straight line and cannot replace the critical assessment of an experienced veterinarian. Finally, the *t* test used for preliminary evaluation and illustration of the differences of the gait parameters PRE-POST does not account for repeated observations due to participation in several event.

5. Conclusion

In the current study, more than two-thirds of elite eventing horses deemed fit to compete showed gait asymmetries exceeding published normal values for head and pelvic movement prior to competition. Strenuous exercise resulted in decreased stride duration, but had no

significant effect on gait asymmetries. It is not known to what extent these asymmetries were pain-related or due to biological variation. This is the first known study that quantified gait asymmetries in elite eventing horses and further research is needed to investigate the impact of these asymmetries on performance.

6. Ethical statement

Informed consent and approval were obtained from the owners and riders of the horses, the eventing committee of the Swiss Equestrian Federation, and the Animal Experimentation Committee of the Canton of Vaud, Switzerland (permit number VD3585).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgements

The authors acknowledge the collaboration of the riders and owners of the horses as well as the eventing commitee of the Swiss Equestrian Federation. We wish to thank Thilo Pfau for his technical support and all the ISME team members and students that helped during data

collection. The study was funded by the ISME research fund. All authors contributed to the conception and design of the study, acquisition of data, analysis and interpretation of data, and preparation of the article. All authors approved the final article.

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Table 1: Quantitative gait analysis was performed on 33 horses before and after the cross-country part of three Concours Complet International events. The distribution of the 140 observations (33 horses, minimum of 15 strides per observation) and whether the observations were below (o) or above (x) the lameness screening threshold set at [14.5]mm for forelimbs and [7.5]mm for hindlimbs are shown

		Event 1		**Event 2**		**Event 3**	
Horse ID	**N**	**before**	**after**	**before**	**after**	**before**	**after**
1	2	o	x				
2	3	o	x	o			
3	4	x	x	x	x		
5	6	o	o	o	o	x	x
6	6	o	o	o	o	x	x
7	5	o	o	x		x	o
8	5		x	x	o	x	x
9	2					x	x
10	6	x	x	o	x	x	o
11	6	o	x	x	x	o	x
12	6	o	x	o	x	o	o
13	4	o	x	x	x		

14	6	x	x	o	o	o	o
15	2			x	x		
16	5	o		o	x	o	x
17	3	o		o	o		
18	6	x	x	o	x	o	x
19	6	o	o	o	o	o	o
20	5	x	x		x	o	x
21	4	o	x			o	o
22	6	x	x	o	x	x	x
23	4			o	o	o	o
24	6	o	o	o	x	o	x
25	3	x	x	x			
27	6	x	o	o	o	x	x
28	3	o	o	o			
29	2	o		o			
30	6	x	x	o	x	o	o
31	2					x	x
32	6	o	o	o	o	o	o
34	2					o	o
35	1					x	
36	1					o	
Total observations		25	23	25	21	24	22

Abbreviation: N = number of observations per horse

Table 2: Descriptive statistics of the absolute values of the gait parameters for the head (HDmin), pelvis (PDmin) and wither (WDmin), and the average stride time (AvStrT) measured in 33 horses before (Pre) and after (Post) the cross-country during the three events

	HDmin (mm)		**PDmin (mm)**		**WDmin (mm)**		**AvStrT (ms)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
N	74	66	74	66	74	66	74	66
Min	-33	-39	-18	-22	-19	-28	598	577
Max	24	26	21	25	10	9	781	724
Median	-2	-1.5	-1	-2	-2.5	-3.5	698.5	680
Mean	-1.9	-3.1	-1.1	-1.15	-2.3	-3.8	691.7	673.2
Std	11.1	15.1	6.5	8.6	5.5	7.0	37.9	33.7
Skew	-0.3	-0.3	0.3	0.5	-0.3	-0.7	-0.4	-0.6
Kurt	3.3	2.3	3.9	3.1	3.6	3.8	2.8	2.8

Abbreviations: N = number of observations; Std = standard deviation; Skew = skewness; Kurt = kurtosis (for a normal distribution the skewness is 0 and kurtosis is 3)

Table 3: Descriptive statistics of the age and weight of the horses as well as of their CK, AST and LA levels measured in 33 horses before (Pre) and after (Post) the cross-country test during the three events

	Age (years)	**Weight (kg)**	**CK (U/L)**		**AST (U/L)**		**LA (U/L)**	
			Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
N	140	140	74	66	74	66	74	66
Range	7 – 18	451 – 631	110 – 457	165 – 513	213 – 629	238 – 598	0.4 – 0.4	3.7 – 22.1
Median	12	529	204	258	310	335	0.4	13.6
Mean	11.5	536	218	278	325	340	0.4	13.7
Std	2.2	37.7	70.3	75.9	74.6	68.5	0	4.5
Skew	0.7	0.3	1.5	1.3	2.0	1.3		-0.2
Kurt	4.3	2.5	5.6	4.3	8.4	4.3		2.4

Abbreviations: CK = Creatine kinase; AST = Aspartate aminotransferase; LA = Lactate; N = number of observations; Std = standard deviation; Skew = skewness; Kurt = kurtosis (for a normal distribution the skewness is 0 and kurtosis is 3)

Table 4: Comparisons of the means of 128 paired observations for the head (HDmin), pelvis (PDmin), withers (WDmin), average stride time (AvStrT), CK and AST before (mo, N = 64) and after (m1, N = 64) the cross-country test of the three events, using two-sample t tests with unequal variances

PrePost	**m0**	**m1**	**m0 – m1**	**P(diff < 0)**	**P(diff ≠ 0)**	**P(diff > 0)**
HDmin (mm)	-2.4	-3.3	0.9	0.65	0.69	0.35
PDmin (mm)	-0.61	-1.05	0.41	0.63	0.75	0.37
WDmin (mm)	-2.73	-3.80	1.07	0.83	0.35	0.17
AvStrT (ms)	695.23	672.09	**23.14**	1.00	**0.00**	**0.00**
CK (U/L)	218.6	280.26	**-61.66**	**0.00**	**0.00**	1.00
AST (U/L)	328.36	341.72	-13.36	0.15	0.31	0.86

Abbreviations: N = number of observations; CK = Creatine kinase; AST = Aspartate aminotransferase. Significant differences are highlighted in bold print

Table 5: Full and final OLS regression models for the head (HDmin) and pelvis (PDmin) gait parameters in 33 horses during the three cross-country events (N = 140)

	HDmin (mm)				**PDmin(mm)**			
	Full model		**Final model**		**Full model**		**Final model**	
	Coef	***P* value**	**Coef**	***P* value**	**Coef**	***P* value**	**Coef**	***P* value**
Sex	2.73	0.478	---	---	0.04	0.984	---	---
Age (years)	-7.26	0.044	-6.45	0.068	-6.25	0.078	-5.81	0.089
Age^2 (years)	0.33	0.013	0.30	0.018	0.28	0.035	0.26	0.043
Weight (kg)	-0.78	0.340	-0.10	0.039	-0.63	0.148	---	---
$Weight^2$ (kg)	0.00	0.414	---	---	0.00	0.148	---	---
Event	-1.79	0.195	---	---	0.10	0.916	---	---
PrePost	10.79	0.226	---	---	-3.78	0.569	---	---
CK (U/L)	0.15	0.177	---	---	-0.08	0.106	---	---
CK^2 (U/L)	0.00	0.102	---	---	0.00	0.106	---	---
AST (U/L)	0.00	0.997	---	---	0.10	0.196	---	---
AST^2 (U/L)	0.00	0.892	---	---	0.00	0.258	---	---
LA (U/L)	-1.99	0.239	---	---	1.04	0.266	---	---
LA^2 (U/L)	0.066	0.311	---	---	-0.05	0.185	---	---
R^2	0.2524		0.1575		0.1765		0.0975	

Abbreviations: OLS = ordinary least square; N = number of observations; Coef = regression coefficient; PrePost = timing of the measurements; CK = creatinine kinase; AST = aspartate aminotransferase; LA = lactate. Statistical significance is highlighted in bold print

Table 6: Full and final OLS regression models for the square root of the withers gait parameter (WDmin) and the average stride time (AvStrT) in 33 horses during the three cross-country events (N = 140)

	WDmin (mm)				**AvStrT(ms)**			
	Full model		**Final model**		**Full model**		**Final model**	
	Coef	***P* value**	**Coef**	***P* value**	**Coef**	***P* value**	**Coef**	***P* value**
Sex	-0.52	0.759	---	---	-0.98	0.906	---	---
Age (years)	4.36	0.144	---	---	12.29	0.381	-5.78	0.015
Age^2 (years)	-0.20	0.079	---	---	-0.77	0.132		
Weight (kg)	-0.05	0.897	---	---	2.61	0.324	0.31	0.006
$Weight^2$ (kg)	0.00	0.974	---	---	0.00	0.364	---	---
Event	0.06	0.916	---	---	11.86	0.002	11.51	0.002
PrePost	3.59	0.549	---	---	7.39	0.710	---	---
CK (U/L)	0.06	0.163	---	---	-0.16	0.487	---	---
CK^2 (U/L)	0.00	0.109	---	---	0.00	0.615	---	---
AST (U/L)	-0.09	0.097	-0.02	0.041	-0.14	0.595	---	---
AST^2 (U/L)	0.00	0.200	---	---	0.00	0.568	---	---
LA (U/L)	-0.78	0.350	---	---	-3.49	0.278	-0.91	0.003
LA^2 (U/L)	0.02	0.437	---	---	0.13	0.295	---	---
R^2	0.1716		0.03		0.4075		0.3496	

Abbreviations: OLS = ordinary least square; N = number of observations; Coef = regression coefficient; PrePost = timing of the measurements; CK = creatinine kinase; AST = aspartate aminotransferase; LA = lactate. Statistical significance is highlighted in bold print

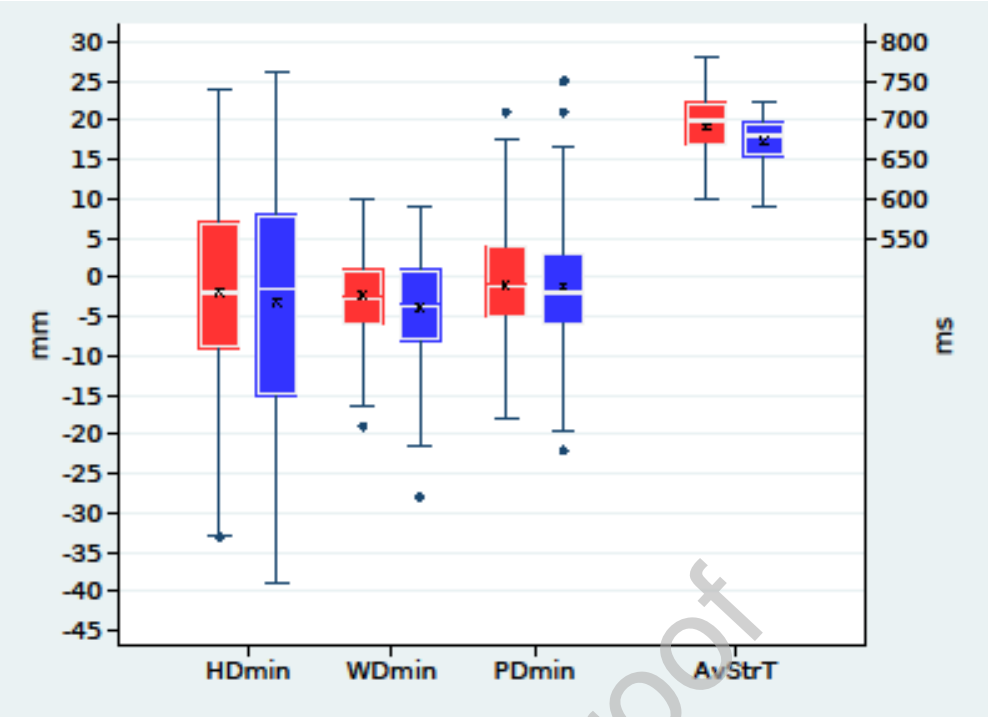

Fig. 1. Boxplot of the three gait parameters measured by a quantitative gait analysis system: the difference between the lowest position of the head (HDmin), withers (WDmin) and pelvis (PDmin) during the mid-stance phase of the left and right limb and of the average stride time (AvStrT) measured before (red) and after (blue) a cross-country test in 33 eventing horses. Symbols: boxes extend from the lower to the upper quartile; whiskers represent 1.5 times the interquartile range above or below the upper and lower quartile respectively; horizontal line marks the median; black crosses indicate means; circles represent outliers.