2nd Dorothy Havemeyer Foundation

Equine Performance Workshop

April 22-25, 2025

Embassy Suites/UK Coldstream

Lexington, Kentucky

Havemeyer Foundation

Scientific/Organizing Committee

Rob Keene

United States – MT

Melissa King

United States- CO

Erica McKenzie

United States – OR

Cris Navas

United States – PA

Allen Page

United States - KY

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Welcome

Welcome to the 2nd Havemeyer Foundation Workshop Equine Performance in Lexington Kentucky.

We are excited to welcome everyone to the workshop at the Embassy Suites, in Lexington Kentucky. This event would not be possible without the generous support of the Dorothy Russell Havemeyer Foundation and Mr. Gene Pranzo. Their tireless and steadfast support of the equine industry through generous support of workshops on various diseases and conditions that affect horse health around the world is special and should not, and is not, taken for granted.

This event is also sponsored by Boehringer Ingelheim Animal Health, Kentucky Equine Research, and Platinum Performance. The quality of the venue and the program would not be possible without the support, and everyone involved. Thank you!

We have two plenary speakers, Dr. Cindy Cole Digitalis Ventures, technical partner and Stephen Smith, founder Kitman Labs, who are highly regarded international experts in their areas of interest. They have graciously taken the time to join us for this workshop, which is tremendously exciting.

We have accepted 33 abstracts for presentation in four areas of interest: injury prevention, wearable technology, cardiology/ respiratory, and performance consideration/other technologies. Many thanks to Melissa King, Erica McKenzie, and Cris Navas who also served on the organizing committee. Our task was to review and accept these abstracts for presentation at this workshop on Equine Performance. We have enjoyed organizing this event and cannot thank all involved enough for their help!

Cheers,

Rob and Allen

Venue, Workshop and Local Information

Venue Address

Embassy Suites by Hilton Lexington/UK Coldstream

1801 Newtown Pike

Lexington, KY 40511, USA

1-859-455-5000

Room check in time is 15:00. Check out time is 11:00.

Badges

Badges will be provided and should be worn at all workshop events. These can be picked up during the Welcome Reception.

A thumb drive with proceedings will be provided. Only agendas will be printed.

Internet

Free Wi-Fi – enter last name and room number.

Meals

Tuesday evening, the Welcome Reception will provide heavy Hors d 'Oeuvres, beer and wine. Breakfast and lunch Wednesday, Thursday and Friday breakfast are all at the Embassy Suites. Wednesday and Thursday dinners will be off site.

Information for Speakers

A laptop and wireless microphone will be available. Please have your presentations, if applicable, loaded at the break prior to your scheduled session. Due to the tight schedule, we ask you to use the PC laptop provided and bring your presentation on a USB drive.

Local Tourist Attractions

Springtime in central Kentucky is a great time to visit. Defender three day even week is ongoing as is racing at Keeneland racetrack. The Kentucky Horse Park is very close to the hotel and has a gift shop and museum. An optional behind the scenes visit to Keeneland racecourse is being planned. It is hosted by Dr Stuart Brown, Vice President of Keeneland Association.

SPONSOR CONTACT INFORMATION

TITLE SPONSOR

The Dorothy Russell Havemeyer Foundation, Inc.

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Attn: Dr. Joe Pagan

Kentucky Equine Research

3910 Delaney Ferry Rd.

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USA

http://www.ker.com

pagan@ker.com

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Program Overview

Tuesday, April 22, 2025

18:30	-	20:30	Welcome Reception at Embassy Suites Hotel

Wednesday, April 23, 2025

Session I. Injury Prevention Chair: Rob Keene

08:30	-	08:40	Welcome and Introductions	Rob Keene/ Allen Page
08:40	-	08:50	**1** EXPLORING UNTARGETED PLASMA METABOLOMICS FOR EARLY DETECTION OF MUSCULOSKELETAL INJURY IN RACEHORSES: INSIGHTS AND CHALLENGES	Maëlle M. Bonhomme
08:50	-	09:00	**2** MUSCLE OXIDATIVE CAPACITY DETERMINATION TO QUANTIFY PERFORMANCE AND TO DETECT EARLY DYSFUNCTION IN SPORT HORSES	Dominique-Marie Votion
09:00	-	09:10	**3** RNA SEQUENCING REVEALS POSSIBLE BIOMARKERS OF FETLOCK INJURY IN RACEHORSES	Joanne Haughan
09:10	-	09:20	**4** INJURY PREVENTION AND PERFORMANCE MONITORING IN EVENTING USING MOBILE HEALTH	Cris Navas
09:20	-	09:30	Q&A Session	
09:30	-	09:50	**5** INJURY PREVENTION STRATEGY IN THOROUGHBRED RACING	Chris E. Kawcak
09:50	-	10:00	**6** THE CALIFORNIA RACEHORSE POSTMORTEM PROGRAM	Francisco A. Uzal
10:00	-	10:10	**7** RACEHORSE EXERCISE HISTORY ASSOCIATED WITH SUDDEN DEATH	Susan M Stover
10:10	-	10:20	Q&A Session	
10:20	-	10:40	Morning Break	
10:40	-	12:00	**P1** PLENARY SESSION BY KITMAN LABS	Stephen Smith
12:00	-	13:00	Lunch	

Session 2. Wearable Technology Chair: Allen Page

13:00	-	13:10	**8** APPLICATION OF WEARABLE TECHNOLOGY TO ACCURATELY QUANTIFY VARIABLES ASSOCIATED WITH PERFORMANCE IN RACIING THOROUGHBRED HORSES	Warwick Bayly
13:10	-	13:20	**9** VALIDATION OF A NOVEL WEARABLE SENSOR TECHNOLOGY IN EQUINE GAIT ANALYSIS	Sandro Colla
13:20	-	13:30	**10** LESION SPECIFIC ALGORITHMS CREATED FROM ACCELEROMETER DATA CAN PREDICT CONDYLAR AND SESAMOID FRACTURES IN RACEHORSES	David H. Lambert
13:30	-	13:40	**11** HOOF-ON AND -OFF DETECTION DURING GALLOP USING A SINGLE INERTIAL MEASUREMENT UNIT	Guillaume Dubois
13:40	-	13:50	**12** COMPARISON OF THREE OBJECTIVE MEASUREMENT SYSTEMS AND TWO SUBJECTIVE EVALUATORS IN NATURALLY OCCURRING EQUINE LAMENESS	Allen Page
13:50	-	14:00	Q&A Session	
14:00	-	14:10	**13** NOVEL METHOD FOR ASSESSING DRESSAGE HORSE TRAINING LEVEL USING GAIT PARAMETERS: A PILOT STUDY	Lise C. Berg
14:10	-	14:20	**14** DAILY TRAINING WORKLOAD OF LOWER-LEVEL JUMPERS: PRELIMINARY RESULTS	Foreman JH
14:20	-	14:30	**15** HOLISTIC USE OF INERTIAL SENSORS FOR DETECTING IMPENDING INJURY IN THOROUGHBRED RACEHORSES IN AUSTRALIA	Ashleigh V. Morrice-West
14:30	-	14:40	**16** SPOTTING INJURY EARLY: TOWARDS IMPROVING THE INTERPRETATION OF SENSOR-BASED MOVEMENT ASSESSMENT IN THOROUGHBRED RACEHORSES	Thilo Pfau
14:40	-	14:50	**17** LOCOMOTION CHANGES INDUCED BY SURFACE FIRMNESS OF A CROSS-COUNTRY COURSE: PILOT STUDY ON 3 HORSES	Nathalie Crevier-Denoix
14:50	-	15:00	Q&A Session	
15:00	-	15:20	Afternoon Break	

15:20	-	16:50	Breakout Discussions and Full Group Discussion re: Wearables + Performance and/or Injury Prevention	Melissa King: facilitator
16:50	-	17:00	Wrap Up Day 1	
17:00	-	18:00	Conclude the day	
18:00	-	21:00	Depart for dinner at Fasig-Tipton sponsored by Boehringer Ingelheim and Platinum Performance	

Thursday, April 24, 2025

Session 3. Cardiology/Respiratory Chair: Cris Navas

08:30	-	08:40	**18** CARDIAC ARRHYTHMIAS DURING INTENSE EXERCISE IN THOROUGHBRED RACEHORSES: INCIDENCE AND ASSOCIATION WITH RACE PERFORMANCE	E. van Erck-Westergren
08:40	-	08:50	**19** EVALUATION OF THE ‘EQUIFIB’ APP WITH POLAR H10 FOR AUTOMATIC ATRIAL FIBRILLATION DETECTION IN HORSES	Gunther van Loon
08:50	-	09:00	**20** FEASIBILITY OF ACQUIRING ECGS REMOTELY AND PREVALENCE OF ARRHYTHMIAS IN THOROUGHBRED RACEHORSES DURING HIGH-INTENSITY WORK	Mary Durando
09:00	-	09:10	**21** EFFECTS OF INDUCED LARYNGEAL HEMIPLEGIA ON AEROBIC CAPACITY AND EXERCISE-INDUCED PULMONARY HEMORRAGE IN THOROUGHBRED RACEHORSES	R. Léguillette
09:10	-	09:20	**22** VALIDATION OF A SMART TEXTILE DEVICE FOR RECORDING ELECTROCARDIOGRAMS IN STANDARDBRED DURING HIGH-SPEED EXERCISE	Sam Franklin
09:20	-	09:30	**23** FEASIBILITY AND APPLICATON OF A SMART PHONE BASED ELECTROCARDIOGRAM FOR SCREENING IN THE THOROUGHBRED RACEHORSE	Jessica Morgan

09:30	-	09:40	**24** USE OF ATRIO SOFTWARE TO ANALYSE ECGS FROM HORSES WITH AND WITHOUT A HISTORY OF ATRIAL FIBRILLATION	Laura Nath
09:40	-	10:00	**25** SHORT BURSTS OF PAROXYSMAL ATRIAL FIBRILLATION DURING EXERCISE IN RACE HORSES: CASE DESCRIPTION, ECG CHARACTERISTICS AND EFFECT ON SPEED PARAMETERS AND SUBSEQUENT PERFORMANCE	F.ter Woort
10:00	-	10:20	Q&A Session	
10:20	-	10:40	Morning Break	
10:40	-	12:00	**P2** Plenary Session	Cynthia Cole Digitalis Ventures
12:00	-	13:00	Lunch	
Session 4. Performance Considerations & Other Technologies Chair: Erica McKenzie				
13:00	-	13:10	**26** OPTIMIZING PERFORMANCE: FITNESS ASSESSMENT IN MARATHON DRIVING HORSES AND PONIES	Carolien Munsters
13:10	-	13:20	**27** A PILOT STUDY OF MOVEMENT ASYMMETRY IN FEI-LEVEL SPORT HORSES: PREVALENCE AND CORRELATION TO PERFORMANCE	Erin K Contino
13:20	-	13:30	**28** ASPECTS OF CONTINUOUS MONITORING OF SKIN SURFACE TEMPERATURE IN ENDURANCE HORSES DURING FIELD EXERCISE	Elisabeth-Lidwien J.M.M. Verdegaal
13:30	-	13:40	**29** INTRAMUSCULAR TEMPERATURE SENSORS FOR MANAGING POST-EXERCISE COOLING IN ATHLETIC HORSES	Michael S. Davis
13:40	-	13:50	**30** EFFECTS OF BLOOD FLOW RESTRICTION TRAINING ON NORMAL EQUINE CARTILAGE AND SUBCHONDRAL BONE	Sherry Johnson
13:50	-	14:00	**31** EFFECT OF DIFFERENT COOLING METHODS ON MUSCLE TEMPERATURE RECOVERY IN EXERCISED TWO-YEAR-OLD THOROUGHBRED RACEHORSES	Joe Pagan
14:00	-	14:10	**32** MECHANICAL NOCICEPTIVE THRESHOLDS AND POSTURAL STABILITY IN HORSES FOLLOWING WHOLE-BODY VIBRATION THERAPY	Katherine L. Ellis

14:10	-	14:20	**33** CONSISTENCY OF CIRCULATING MYOSTATIN IN THOROUGHBREDS GENOTYPED FOR A SINE INSERTION IN THE MYOSTATIN GENE PROMOTER	Katherine Hanousek
14:20	-	14:40	Q&A Session	
14:40	-	15:00	Afternoon Break	
15:00	-	16:30	Break Out Sessions and Full-Group Discussion	
16:30	-	17:00	Closing Summary and Future Action Plan	Melissa King/Cris Navas facilitators
17:00	-	17:15	Conclude for day	
17:15	-	21:30	Bus Leaves Embassy Suites promptly at 17:30 to Depart for dinner and tour at Kentucky Equine Research	

Friday, April 25, 2025

08:15 - 10:00 Depart on your own - Optional Morning Keeneland Tour
Dr. Stuart Brown VP Equine Safety – Keeneland Association

SESSION I. Injury Prevention

1

EXPLORING UNTARGETED PLASMA METABOLOMICS FOR EARLY DETECTION OF MUSCULOSKELETAL INJURY IN RACEHORSES: INSIGHTS AND CHALLENGES

Maëlle M. Bonhomme[1], Ulrike Brüning[2], Janine Wiebach[2], Florence Patarin[1], Anne Couroucé[3,4], Claire Leleu[5], Eric A. Richard[4,6], Jennifer A. Kirwan[2], Dominique-Marie Votion[1]

[1]University of Liège, Faculty of Veterinary Medicine, Department of Functional Sciences, FARAH, Liège, Belgium; [2]Berlin Institute of Health (BIH) at Charité Universitätsmedizin, BIH Metabolomics, Berlin, Germany; [3]Oniris, National Vet School of Nantes, Equine Department, Nantes, France; [4]University of Caen Normandie, Biotargen, Saint Contest, France; [5]Equi-Test, Grez-en-Bouère, France; [6]LABÉO (Frank Duncombe), Saint Contest, France

Musculoskeletal injuries (MSIs) in racehorses present major challenges to animal welfare, performance, jockey safety and economic sustainability, especially in jump racing. While injuries are an inherent risk in any sport, most MSIs are not sudden events but rather the result of progressive, subclinical tissue deterioration that often precedes clinical expression. Identifying early warning signs could allow racing stakeholders to adjust training or racing schedules in time to prevent injuries and mitigate their impact. Effective prevention strategies are therefore essential to ensure the sustainability of athletic activities.

We hypothesize that the development of MSIs during training is associated with early, measurable changes in blood parameters related to inflammation, mitochondrial function, oxidative stress, energy metabolism and / or tissue remodeling. Untargeted metabolomics, with its ability to profile a broad range of metabolites across diverse pathways without predefined assumptions, offers a powerful approach to uncover subtle biochemical alterations indicative of subclinical injury risk. This study aimed to explore untargeted metabolomics as a tool for identifying early blood biomarkers associated with MSI development during jump racehorses' training.

To accomplish this an 18-month longitudinal field study was conducted to monitor jump racehorses in training across three stables in France between September 2022 and February 2024. Among other parameters, resting, and pre-feeding blood samples were collected every two months under strict pre-analytical conditions (*i.e.*, immediate centrifugation, replication into aliquots and immediate -80°C storage). Prior to inclusion, trainers and stable veterinarians confirmed the fitness of each horse for training. Due to the dynamic nature of training, involving alternating phases of rest and active work, no horse was monitored continuously throughout the study. New horses joining the stables during the protocol were included in the follow-up. On average, horses participated in three consecutive visits, equivalent to a six-months monitoring period. All horses within each stable were maintained under consistent conditions, trained by the same trainer and provided with a uniform diet. In total, 164 jump racehorses were monitored during the study period, with injury tracking identifying 46 horses (28%) that sustained MSIs (*i.e.*, horses out of training for more than 21 days, supported by, either a diagnosis by a veterinarian or clinical signs described by trainers). Among injured horses, 2% experienced muscular pathologies, 4% ligament injuries, 4% combined ligament and bone lesions, 15% bone lesions excluding bone fractures, 15% bone fractures and 59% tendon injuries. Among the bone cases, four fractures were catastrophic, resulting in euthanasia.

For this exploratory study, horses with bone fractures at various anatomical sites (e.g., scapula, third metacarpal bone, first phalanx, proximal sesamoid bone, tibial lateral malleolus and third metatarsal bone; n = 7; "bone cases") and six horses with superficial digital flexor tendon lesions (n = 6; "tendon cases") were recruited. Each injured horse was matched to a control horse that remained fit to race throughout the study. Matching criteria included stable of origin, sampling day, training and racing schedules and, where possible, sex, age and breed. Due to logistical constraints, blood samples could not be collected just prior to the injury; therefore, analyses focused on the most recent samples collected prior to injury expression. At least one sample was collected within two months before the injury occurrence, with some pairs having samples collected up to eight months prior to injury. Analyses were conducted separately for bone fractures and tendon injuries.

Routine biochemical parameters, including GGT, succinate dehydrogenase (SDH), glutamate dehydrogenase (GLDH), AST, ALP, albumin, creatine kinase (CK), cholesterol, serum amyloid A (SAA), bilirubin and bile acids, were measured using photometry (Antech Diagnostic Laboratory, Liège, Belgium). Additionally, untargeted metabolomics analyses were conducted to identify potential metabolic signatures indicative of subclinical injury. Metabolomic profiles of injured horses and their matched controls were compared for both the bone fracture and tendon studies.

Untargeted metabolomics was performed using ultra-high-performance liquid chromatography (Vanquish Flex UHPLC systems, Thermo Fisher Scientific, USA) coupled with high-resolution mass spectrometry (Orbitrap Exploris 240 mass spectrometer, Thermo Fisher Scientific, USA) in both positive and negative ionization modes. Tandem mass spectrometry was applied in a data dependent fashion, and stringent quality control measures were implemented to ensure robust data processing.

Group characteristics were compared using the Wilcoxon rank-sum test for continuous variables, Fisher's exact test for categorical variables and a Binomial test to evaluate deviations from equal proportions. Differences in blood markers were assessed through univariate and multivariate analyses. Univariate analyses included paired Wilcoxon rank-sum tests at the final sampling time point and non-parametric two-factors longitudinal analyses (nparLD package in R). Blood markers were considered relevant if they exhibited significant differences between cases and controls, maintained these differences over time, or showed a distinct temporal patterns. Multivariate analyses, including unsupervised Principal Component Analysis (PCA) and supervised Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA), were used to identify potential injury-related features.

The list of potential features of interest was derived by combining results from univariate (*p-value* not corrected for multiple testing < 0.05) and multivariate analyses (OPLS-DA model VIP score > 1), acknowledging the exploratory nature of the study and the limitations in statistical rigor. PERMANOVA (Permutational Multivariate Analysis of Variance) was performed on the distance matrix calculated from the PCA scores (*e.g.*, Euclidean distance), to evaluate the associations between metabolomic profiles and metadata variables. Data visualization included PCA plots and hierarchical clustering heatmaps, providing insights into grouping patterns.

No significant differences were observed between the bone and tendon studies regarding age, breed, gender, trainer, previous racing experience, number of starts during the study period, number of samples collected, or the sampling day corresponding to the last sample (*p-value* > 0.05). However, a significant difference was identified in the interval between the last sample collection and injury expression among cases (*p-value* = 0.018), with tendon cases exhibiting a shorter interval (18 ± 17 days) compared to bone cases (43 ± 13 days). In the bone study, 42.9% of injuries occurred during training and 57.1% during racing. Interestingly, 42.9% were triggered by jumping and 57.1% occurred during flat gallops. In contrast, all tendon injuries were diagnosed during training and were not directly associated with a fence. At two months before injury, no individual biochemical parameter significantly distinguished cases from matched controls in either study. However, some temporal fluctuations were observed in parameters such as ALT, GGT, total bilirubin, indirect bilirubin and SDH, independent of injury status (*p-value* < 0.05), suggesting they may reflect physiological variations over time rather than injury-specific changes. Metabolomics profiles were dominated by changes influenced by stable (*i.e.*, environment, diet, training management, etc.) and sampling day, although both univariate and multivariate statistical analyses enabled the selection of a set of features potentially distinguishing cases and controls prior to injury. Features selected in the tendon study demonstrated clearer discrimination compared to the bone study, potentially due to higher biological variability in the bone cohort (various anatomical sites affected). Additionally, negative ionization mode provided more information than positive mode in both the bone and tendon studies. While definitive identification of the selected features was not achieved due to the absence of tandem mass spectrometry data for those particular species, the mass spectrometry data provide a foundation for further targeted analyses and refined biomarker discovery approaches.

This exploratory untargeted metabolomics study underscores the potential for early MSI detection in racehorses while emphasizing the critical need to address for confounding factors, account for individual variability and enhance feature identification methods to improve injury prevention strategies in equine sports medicine.

2

MUSCLE OXIDATIVE CAPACITY DETERMINATION TO QUANTIFY PERFORMANCE AND TO DETECT EARLY DYSFUNCTION IN SPORT HORSES

Dominique-Marie Votion

University of Liège, Faculty of Veterinary Medicine, Department of Functional Sciences, Comparative Veterinary Medicine, FARAH, Liège, Belgium

The athletic performance of horses is influenced by inherited physiological traits and the structural and functional adaptations of various systems to training. Maximal oxygen consumption (VO_{2max}) is a recognized marker of athletic capacity, integrating the oxygen transport and utilization efficiency, from inspired air to mitochondria, for energy production. However, VO_{2max} is primarily limited by oxygen delivery, as muscle oxidative capacity surpasses cardiovascular transport capabilities. Therefore, direct assessment of muscle mitochondrial oxidative capacity should provide a more precise evaluation of aerobic fitness and athletic potential.

The objectives of the studies performed was (1) to demonstrate the feasibility of using high-resolution respirometry with minimally invasive muscle microbiopsies to assess mitochondrial oxidative capacity in horses, (2) to investigate the influence of fitness level and training status of sport horses on mitochondrial oxidative capacity, (3) to evaluate high-resolution respirometry as a tool for monitoring training adaptations, (4) to determine whether muscle oxidative capacity correlates with performance outcomes, and (5) to explore the potential of high-resolution respirometry to detect early mitochondrial dysfunction that can lead to poor performance and/or myopathies.

Muscle microbiopsies were collected from the *triceps brachii*, selected for its propulsive and postural roles, using a semi-automatic 14 G needle. Approximately 20 mg of muscle tissue per horse was analyzed using high-resolution respirometry, employing multiple substrate-uncoupler-inhibitor titration protocols designed by us specifically for equine muscle.

First, validation of standardized methods with 32 horses of different breeds (14 Arabian, 4 Crossbred Arabian, 6 Half-Blood and, 8 Standardbreds), and fitness levels (3 overweight, 8 untrained, 16 trained and, 5 competitive) confirmed feasibility and reproducibility of this approach. The microbiopsy technique overcomes limitations of traditional muscle biopsy by requiring small tissue samples (about 2 mg of tissue per analysis), enabling routine evaluation even during competitive seasons. In addition, we demonstrated that muscle samples preserved in specialized media remained viable for mitochondrial analysis for up to four days, enabling field-to-laboratory transport.

Secondly, results showed that muscle oxidative capacity is closely associated with training status and fitness levels across disciplines, including endurance, jumping, eventing, and dressage. For both studies, the Wilcoxon signed rank and Mann-Whitney-Wilcoxon tests were used to compare paired and unpaired data, respectively.

Third, longitudinal studies on 8 endurance horses revealed significant increases in muscle oxidative capacity following training, as assessed by a one-way ANOVA for repeated measurements among the different sampling times for training effects, with post hoc comparisons of means provided by Tukey's least significant difference test.

Fourthly, applying the technique on 10 Arabian horses from the French national endurance team, a strong correlation between muscle oxidative capacity and race performance was observed. Horses with the highest muscle oxidative values achieved top race rankings and faster average speeds (R^2 = 0.76 and R^2 = 0.74, respectively, $p < 0.001$), as determined by linear regression analysis to assess the relationship between oxymetric values and ranking or speed. Notably, the horse with the highest muscle oxidative value earned the "best-conditioned horse" prize at the World Equestrian Games at Lexington. Finally, beyond performance prediction, high-resolution respirometry identified subclinical mitochondrial dysfunction in underperforming horses, both in endurance and trotter horses, enabling early detection of exercise-induced myopathies.

In conclusion, high-resolution respirometry appears as a powerful tool for quantifying muscle oxidative capacity, predicting athletic performance, and identifying early signs of muscle pathology in sport horses. Its applications range from optimizing training strategies to enhancing safety and performance in equine athletes. Repeated high-resolution respirometry assessments may be used to track the functional recovery of horses, informing decisions about safe re-engagement in competition. The technique has the potential to offer a deeper understanding of the physiological determinants of performance and health in sport horses.

3

RNA SEQUENCING REVEALS POSSIBLE BIOMARKERS OF FETLOCK INJURY IN RACEHORSES

Joanne Haughan[1], Lauren Pittman[1], Daniel Beiting[2], Kyla Ortved[1], Mary Robinson[1,3]
[1]Department of Clinical Studies-New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Pennsylvania, USA; [2]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Pennsylvania, USA; [3]Pennsylvania Equine Toxicology and Research Center, West Chester University, Pennsylvania, USA.

Repetitive stress injury to bone during training can cause changes in the equine fetlock joint visible on CT images. In Thoroughbred racehorses, these changes can lead to joint degeneration and can be associated with future condylar fractures. CT imaging is considered the "gold standard" for viewing early changes in bone and is used in some racing jurisdictions to screen and withdraw racehorses prior to high profile races to reduce the risk of catastrophic injuries. However, this imaging modality is expensive, requires sedation and is only accessible at a limited number of veterinary clinical institutions worldwide. Identification of biomarkers in blood samples could provide a less expensive, less-invasive alternative that could be used to screen larger numbers of racehorses in the field and help determine which horses should be referred for advanced imaging prior to racing. This study aimed to use RNA sequencing to identify gene expression signatures that could be possible biomarkers of fetlock injury in Thoroughbred racehorses.

Six two-year-old Thoroughbred racehorses in their first year of training were selected from a larger cohort for this study[1]. Three horses had minimal evidence of fetlock pathology ("H" horses: 1 filly, 1 gelding, 1 colt) and three horses developed more severe fetlock pathology ("P" horses: 2 fillies, 1 colt) after 6 months of race training as diagnosed on standing cone beam CT imaging by a board-certified veterinary radiologist and a board-certified large animal surgeon. Blood samples were taken at 0 and 6 months after enrollment, concurrent with CT images and processed immediately. RNA was extracted from WBCs, isolated from the buffy coat, using TRIzol® followed by chloroform extraction and isopropyl alcohol precipitation and then air dried, dissolved in nucleotide-free water and stored at -80°C until further processing. Thawed RNA samples were treated with RNase-free DNase I to remove genomic DNA. RNA quality and quantity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). Sequencing libraries were prepared using the Illumina TruSeq Total Transcriptome kit (Illumina). Samples were sequenced on an Illumina NextSeq 2000 to produce 118 bp single end reads. Raw reads were mapped to the equine reference transcriptome (Ensembl; EquCab 3.0) using Kallisto, version 0.46.1. Further analysis was carried out using R version 4.4.1 in RStudio version 2024.04.2 and Bioconductor, version 3.20. The TxImport package was used to summarize transcript data at the gene level. Data was filtered, removing genes with <1 log2 counts per million in at least 3 samples and then normalized using the TMM method from EdgeR. Principal component analysis (PCA) was carried out on normalized, filtered data using the prcomp function. Using the limma package, data were variance-stabilized with voom and differentially expressed genes were identified with linear modeling (FDR ≤ 0.05; absolute logFC ≥ 1), after correction for multiple testing with Benjamini-Hochberg. Gene set enrichment analysis (GSEA) was carried out on all identified genes across samples using the GSEA function in clusterProfiler and the MsigDB 'C2' canonical pathways collection.

A total number of 526.3 million reads were generated from all 12 samples. The mean number of reads per sample was 43.86 million +/- 2.84 million. On average (mean +/- SD), 72.3% +/- 0.6% of the reads in each sample were pseudoaligned to the equine reference genome by kallisto, generating abundance data for 49,882 +/- 402 transcripts per sample which could be summarized to 13,868 +/- 42 genes per sample. After filtering and normalization, PCA showed that samples clustered by disease status (Fig. 1A) but not by time point (Fig.1B). Eight genes were differentially expressed between H and P horses (Fig. 2A). Two genes were significantly upregulated, and six genes were significantly down regulated in P horses compared to H horses (Table 1). GSEA revealed 30 gene sets that were differently enriched in P

[1] Ciamillo SA, Wulster KB, Gassert TM, Richardson DW, Brown KA, Stefanovski D, Ortved KF. Prospective, longitudinal assessment of subchondral bone morphology and pathology using standing, cone-beam computed tomography in fetlock joints of 2-year-old Thoroughbred racehorses in their first year of training. Equine Vet J. 2025 Jan;57(1):126-139. doi: 10.1111/evj.14048. Epub 2024 Jan 21. PMID: 38247205.

and H horses (Figure 2B) indicating differences in gene expression related to key physiological processes between the two groups.

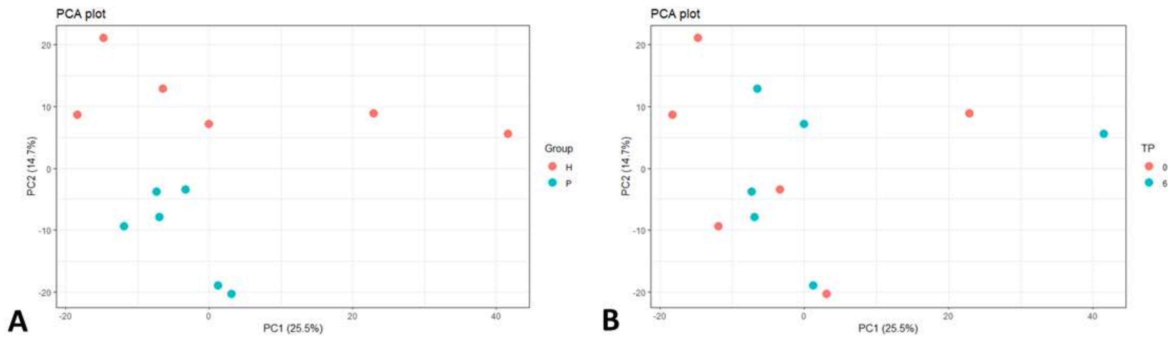

Figure 1. Principal component analysis (PCA) showing PC1 and PC2 for RNA-seq data from H horses (n=3) and P horses (n=3) at 0 and 6 months after enrollment. Samples are colored according to **A:** disease status group (H/P) and **B:** timepoint (TP).

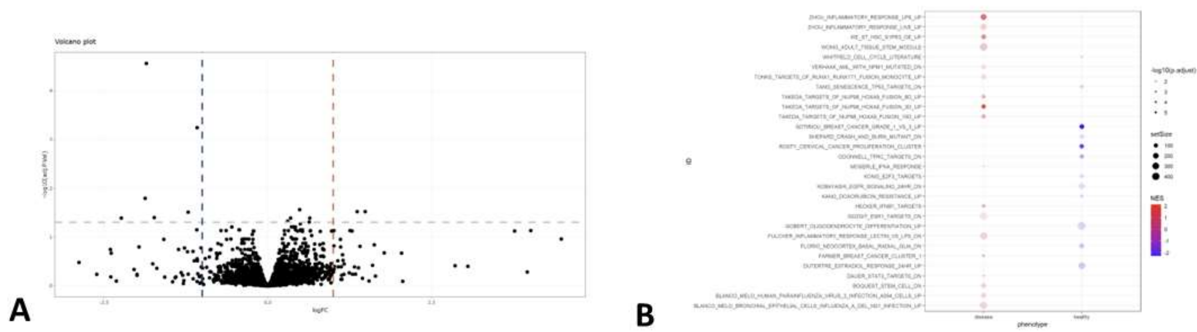

Figure 2. A: Volcano plot of differentially expressed genes (black dots) between H and P horses. Genes above the grey line (FDR <0.05) are significantly differentially expressed (blue line: logFC = -1, red line: logFC = 1). **B:** Bubble plot showing significantly enriched gene sets in P (disease) and H (healthy) horses.

Table 1. Differentially expressed genes (FDR<0.05, logFC >1) between P and H horses (logFC = log Fold Change, adj. pval = adjusted *P* value (FDR)).

Gene Symbol	Function	logFC	*adj. pval*
OLR1	enables low-density lipoprotein particle receptor activity	1.49	*0.030*
PRRG1	enables calcium ion binding	1.37	*0.030*
RNF212B	enables SUMO transferase activity	-1.08	*0.001*
SKP2	enables Protein binding	-1.22	*0.031*
RANBP3L	enables GTPase activator activity	-1.73	*0.040*
TTN	enables ATP binding, protein binding, protein kinase activity and structural constituent of muscle	-1.85	*0.000*
CYBRD1	enables oxidoreductase activity	-1.87	*0.016*
EHD2	enables GTP binding, enables calcium ion binding, enables protein binding	-2.24	*0.041*

In conclusion, RNAseq identified 8 differentially expressed genes in circulating WBCs from thoroughbred racehorses that developed fetlock pathology compared to horses that did not during the first 6 months of race training. Furthermore, GSEA revealed more widespread differences in gene expression between the two groups. Additional studies in a larger population of racehorses are necessary to identify other genes of interest as well as to validate the ability of the genes identified in this study to predict the presence of fetlock pathology in a larger population of Thoroughbred racehorses.

4 INJURY PREVENTION AND PERFORMANCE MONITORING IN EVENTING USING MOBILE HEALTH

Cris Navas[1], Kai Wang[2], Kaja Walter[2], Phoebe Smith[3], Mary Durando[4], Guillaume Dubois[5], Mutian Niu[2]

1 University of Pennsylvania, College of Veterinary Medicine, Clinical Studies New Bolton Center, PA, USA. 2 ETH Zurich, Zurich, Switzerland, 3 Riviera Equine CA, USA. 5 Arioneo, Equimetre, Paris, France. 4 Equine Sports Medicine Consultants, DE, USA.

The long-term goal of this research is to establish a performance monitoring, injury, and exercise-associated death prevention program for equine athletes, using mobile health technology. The information we present here was obtained to seek preliminary associations of candidate variables with injury and performance in Eventing horses and to inform a large-scale study.

A cohort study was designed to longitudinally monitor Eventing horses in active training and competition. Institutional Animal Care and Use permission and Privately Owned Animal Consent Protocols were obtained. Horses were ridden following their usual routines. The fitness tracker (Arioneo Equimetre®, Paris, France) records a one-lead electrocardiogram and data from GPS and motion sensors (accelerometer, gyroscope, and magnetometer). From these direct outputs, variables that describe internal and external training loads are calculated. The type of workout was classified as 'Cross Country Schooling', 'Flat', Gallop', 'Hack', 'Jumping', 'Trotting Set', 'Cross Country Competition' and 'Water' following the type of workouts reported by the riders enrolled in the study. Riders were asked monthly to report injuries, illness, and good/bad performance during competitions. A horse with good performance was defined as one that finished in the top 20% of a 3* FEI-sanctioned competition or an intermediate level or higher USEF-sanctioned competition. Elite horses were defined as horses that finished in the top 5 of 4* or 5* level FEI-sanctioned competitions or advanced level USEF-sanctioned competitions. Average horses were defined as horses that did not meet the definition for injured, good, or elite classifications.

Summary statistics of normal values of the selected variables were calculated according to data distribution. Differences between levels were analyzed within each training type. Density plots by level were made and inspected. All measured variables were then fitted into a linear mixed model with level as a fixed effect and horse nested in the rider as a random effect. Models were fitted within each training type. Since level and performance overlapped, the difference between performance was analyzed within each level and within the training type. Selected a priori hypotheses were tested prospectively and Post hoc observations were made when potentially relevant differences between levels or performance groups were identified. Significance was set at $P<0.05$. The a priori hypotheses were made based on observations previously made in the literature, observation of riders or were included to answer questions that the investigators considered useful to plan future studies. Examples of these hypotheses are: 1- Heart rates are different in different types of workouts. 2- Heart rates during recovery are different in different types of workouts. 3- Horses in higher levels show faster heart rate recovery times than horses in lower levels. 4- The stride length during 'Gallops' is longer in horses of higher levels. 5- The stride rate and symmetry of the trot are higher, and stride length is longer in warmups of horses of higher levels, non-injured and more successful horses. 6- A higher heart rate during the warmup prior to a 'Gallop' workout is found in injured and less successful horses. 7-A higher heart rate during the recovery period after gallops is found in injured horses and less successful horses.

Fifty-three Eventing horses ridden by 21 professional and amateur Eventing riders collected 1466 exercise recordings. Age was (mean±SD) 10±3 years old, and there were 9 mares and 44 geldings. Six were Thoroughbreds, 46 were registered Warmbloods, and 1 was a Thoroughbred Cross. Preliminary significant ($p<0.05$) findings were that: 1- heart rates are different in different types of workouts; 2- absolute heart rates and % of peak heart rate during recovery are different in different types of workouts; 3- the maximal stride length, stride length at 330m/min, and stride length at 550m/min during 'Gallops' is longer in horses of higher levels; 4- the stride rate and symmetry of the first trot are higher in 'Gallops' of non-injured and more successful horses but this was not consistent across levels; 5- The maximal stride length, stride length at 330m/min, and stride length at 550m/min during 'Gallops' is longer in non-injured and more successful horses, but this was not consistent across levels; 6- The mean speed of the first trot is higher in injured and less successful horses and this was achieved by a higher frequency and shorter length of the strife; 7- A higher heart rate during the warmup prior to a 'Gallop' workout is found in injured and less successful horses but this was not consistent across levels.

The most relevant conclusion is that there were several variables that were different between levels, performance, and injury groups. Examples of variables that emerged as potentially useful for the development of an injury prevention and performance monitoring program and are easy to communicate to riders are in the table below.

	Injury concern	Values in Elite horses
Heart rate during the warmup trot	>115/min (NA in advanced)	NA
Symmetry during the warmup trot	<70%	>80%
Stride rate during the warmup trot	>1.4 strides/sec	<1.3 strides/sed
Stride length at a speed of 333m/min	<3.3 m	>3.4 m, most common >3.6 m
Stride length at a speed of 550m/min	<4.7 m	>4.9 m
Heart rate 2 mins after a gallop	NA	<115/min
Heart rate 5 mins after a gallop	NA	<45% peak heart rate

The main limitation of the study is that the size of the study group does not allow to account for the large variability in the reasons behind performance and injury. Subclinical or previous diseases could affect fitness tracker values, and these were heterogenous in the study group. Setting, rider talent and background, footing, horse age, breed, goals, weather, and incline/gradient during gallops were all heterogeneous and may confound the results. The many comparisons increase the risk of type 1 error, and results can only be used to plan for future larger research programs. Refined classifications in more targeted larger studies may help answer questions regarding performance and injury and the current study serves as a proof of principle for an approach to injury prevention using mobile health.

5

INJURY PREVENTION STRATEGY IN THOROUGHBRED RACING

Chris E. Kawcak

Translational Medicine Institute, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA

In June 2024, the International Federation of Horseracing Authorities (IFHA) convened a meeting of a global group of researchers, clinicians, and regulators to develop a strategic plan to translate current and future research into actionable items for use by the Thoroughbred (TB) racing industry. The primary goal was to develop realistic screening programs to identify the horse at risk of injury.[1] The purpose of this report is to review the strategy for discussion for other equine industries. The global transport of competition horses today requires some form of unity across federations. During the IFHA meeting it was concluded that global decision-making must be balanced by local factors that reflect “inter-jurisdictional differences”.

In TB racing the pathogenesis of most injuries are known to be the result of chronic fatigue processes. Although less characterized in sport horse and western performance horse disciplines, the pathogenesis of injuries is felt to be similar in some instances, as diagnostic imaging often shows indications of chronic, pathologic changes, and many horses show performance and behavioral changes prior to onset of clinical injury.[2] However, individual variation in disease progression and the difficulty in detecting subtle pathologic changes makes it necessary to use “secondary indicators” of tissue fatigue to detect horses approaching clinical injury.[1] Workload (volume and intensity), physiologic response to exercise and behavior are now commonly being used in human sport to manage individual athletes. The same is being investigated in the TB racing industry. Training and competition surfaces are modifiable and optimum surface qualities have been investigated. This type of work has begun in sport horse disciplines, but more investment in this work is needed to give equine athletes consistent surfaces to which their tissues could adapt to.

Exercise intensity has been shown to be a factor in TB racehorse injury, and the volume of high-speed work has been clearly associated with fracture. But making a recommendation for volume of high-speed work is challenging considering the individual variation in work required to maintain tissue strength and that which can cause progressive damage. The translation of this philosophy to sport and western performance horses is even more difficult considering the wide range of loads and work volumes needed to achieve and maintain tissue strength. Initial efforts to characterize workload volume and intensity has led to changes in practice, as in Racing Victoria, they have found that trainers who put their horses through lower volume of high-speed work have reduced their incidence of catastrophic injury without compromising performance.[3] More work is needed to verify these results, but the goal of reducing injury while optimizing performance seems achievable.

Practical methods that can help to identify the horse at risk of injury will likely involve multiple data sources that are aggregated, integrated and analyzed based on the discipline and individual. For instance, genetic factors have been associated with fracture in the TB racehorse, yet the application of that body of work to a specific horse is impossible at this point. It might be more practical to use this information to apply greater scrutiny or specific parameters to their management. In human sport, variability in gender, age, race and sport type are all factors that determine the type of data used to prevent injury and optimize performance. The same processes could be used in equine athletes. Mare and foal health and activity can also be managed to optimize athletic health.[4] Serum biomarkers also have potential to be used as monitoring tools. To date the results of these studies are mixed, but in practice the information can be valuable as more data are acquired. Epidemiologic factors can be helpful and are currently being used to screen TB racehorses in several jurisdictions – although how that is being done is not completely clear. Wearable technology is gaining popularity, and at this time characterization of workload volume and changes in stride characteristics have been shown to be associated with injury.[5,6] Association is valuable, but prediction (a much more difficult outcome to reliably achieve) is the goal.[7] Wearable technology must be as unobtrusive to a training program as possible in order to capture the required data to make it useful. Since these data have been associative at best, deviation from normal for a particular athlete over time will likely be needed. To progress from association to detection to prediction will take massive amounts of data, acquired regularly, in a uniform manner by a collaborative industry.[7] This will be a much more difficult thing to do in the equine industry compared to human sport based on limited numbers and resources.

Layered on top of these technologies are advanced diagnostic imaging, which brings a significant financial burden as a screening tool. Pre-race CT screening for the Melbourne Cup and PET imaging at Santa Anita have made significant impacts on the incidence of catastrophic injuries, but the cost and elimination of some horses that might have otherwise raced safely continue to be factors that limit their use and open them up for criticism. It is likely that advanced diagnostic imaging is a tertiary level of screening after primary (wearable, biomarker, etc as above) and secondary (clinical examination) identify the need for further characterization of an individual.

Even with this information, the relatively low incidence of injury adds to the complexity of building predictive models. For TB racing, the plan is to test in series to remove horses at each level, thus reducing the denominator to artificially increase prevalence. To date, this technique has shown promise when used by the Hong Kong Jockey Club. Broad collaboration will be needed to continue this type of work.

Regardless of how well a predictive model can work, and how quickly and accurately it can be iteratively updated, injury will still occur. As such, industries and society should at least discuss risk tolerance. Low risk tolerance can result in more expensive screening protocols and higher athlete scratches from competition, high risk tolerance could result in more injuries that might have been prevented. The monetary impact of injury in human sport is often openly discussed and brought into operational strategy, especially where the impact can be significant.

Given this information, the research, clinical and regulatory group brought together at the IFHA meeting last May decided on the following goals: 1. Develop unity on defining injury and injury outcomes to draw comparisons between groups; 2. Validate and standardize historical data and data collection protocols; 3. Ensure access to advanced diagnostic imaging; 4. Develop frameworks for horse profiling systems; 5. Global collaboration facilitated by industry to enhance longitudinal studies; 6.Research on training methods; 7. Development of a global biobank; 8. Development of a research repository; 9. Funding and administrative support for a global research pool, especially for the development of young, talented early career researchers; 10. Development of data collection tools where needed. Consideration of these strategies within other disciplines could help to unify efforts to reduce injury, improve welfare, optimize business models and facilitate open discussions within society about the role of horses in sport.

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6

THE CALIFORNIA RACEHORSE POSTMORTEM PROGRAM

Francisco A. Uzal,[1] Jeff Blea,[2] Sue Stover,[3] Carlos Schild[1]

1-California Animal Health and Food Safety Laboratory and E, School of Veterinary Medicine, University of California-Davis, 2-California Horse Racing Board, 3-J.D. Wheat Veterinary Orthopedic Research Laboratory, School of Veterinary Medicine, University of California-Davis

The California Horse Racing Board (CHRB) in partnership with the California Animal Health and Food Safety Laboratory System (CAHFS) has been administering a post-mortem program for all race horses that die spontaneously or are euthanized in a facility under the jurisdiction of the Board, since 1990. The objectives of the program are: 1) to determine the nature of injuries occurring in racehorses, 2) to determine the reasons for these injuries, and 3) to develop injury prevention strategies. To accomplish this, a broad, cooperative approach was developed, including CAHFS performing the necropsies, CHRB providing funding for postmortem examinations and ancillary testing, and the racing associations providing transportation of the horses to the nearest laboratory facility. Regardless of the manner or cause of death, a full necropsy is performed on every horse, which includes examination of all systems and, in many cases, performing histopathology and multiple ancillary tests (e.g. bacteriology, virology, parasitology, toxicology, etc.). In the cases of catastrophic musculoskeletal injuries of the limbs, both the affected and contralateral legs are examined, with special emphasis on detection of pre-existing lesions. In addition to musculoskeletal injuries, medical causes of disease and/or death of racehorses (colic, pneumonia, etc.), are also studied. Reports are produced in a comprehensive, yet user-friendly manner, including diagrams and photographs of the main lesions. Information from the necropsy reports is analyzed by CHRB in an effort to elucidate the specific cause of catastrophic injuries or other causes of death. This information is also used by CHRB to educate equine veterinarians, trainers, stewards and owners in several ways, including fatality reviews.

Through June 2024, 7614 necropsies of racehorses have been performed as part of the CHRB-CAHFS post-mortem program. A peak of racehorse fatalities occurred in 2006, with a total of 325 fatalities. From that year on, the number of fatalities showed a marked and steady decline until the fiscal year 2022-2023, with a total of 65 fatalities. A modest increase was observed in 2023-2024, with a total of 87 fatalities. Traditionally, musculoskeletal injuries were responsible for approximately 70-75% of the race horse fatalities in California, with the remaining cases associated with medical conditions (e.g. colitis, pneumonia, encephalitis, etc.). However, with the recent reduction in musculoskeletal injuries, this rate has been progressively declining until the fiscal year 2022-2023, when for the first time, the percentage of medical cases (52%) was higher than the percentage of musculoskeletal injuries (48%). Results from the 24 years of the CHRB-CAHFS necropsy program indicate that approximately 90% of catastrophic musculoskeletal injuries are associated with pre-existing injuries, the great majority of which can be resolved if diagnosed in a timely fashion. The main lesson learned from these results is therefore, that the great majority of catastrophic injuries of racehorses are preventable.

Amongst the medical cases, the number of sudden deaths remain more or less stable (10-12 cases a year). The cause of more than 50% of these deaths remain undetermined, and this constitutes the next big challenge for the CHRB-CAHFS necropsy program and the racehorse industry in general.
A thorough and comprehensive post-mortem program is critical to determine and understand the cause of injury and death of racehorses and develop prevention strategies. This has been clearly demonstrated by the California experience and hopefully will be supported by the mandatory necropsy program recently mandated by the Horseracing Integrity and Safety Authority at the national level in the US.

7

RACEHORSE EXERCISE HISTORY ASSOCIATED WITH SUDDEN DEATH

Susan M Stover[1], Francisco A Uzal[2]

[1]JD Wheat Veterinary Orthopedic Research Laboratory, Dept. VM:SRS, One Shields Avenue, University of California, Davis, Davis, CA 95616 USA and [2]California Animal Health and Food Safety Laboratory System, University of California, Davis, CA USA

The etiopathogenesis of exercise associated sudden deaths in racehorses is largely unknown. An international multicenter study of post mortem findings in racehorses that died suddenly determined a definitive cause of death in only 53% of cases, with cardiac, pulmonary, vascular, skeletal, and nervous system affected.[1] The challenge is to identify racehorses at risk for sudden death so diagnostic examinations and preventive strategies can be implemented. When compared to a large cohort of unaffected horses, exercise history variables have been identified among potential risk factors for sudden death. In the U.K., increasing race distance and decreasing number of race starts in the previous 1, 2, 3, 6, and 12 months were associated with increased risk.[2] In the US, fewer career starts and fewer starts in the previous 1 month, and having had a lay-up were associated with increased risk for sudden death.[3] In a case-control study comparing fatalities between horses that had sudden death due to cardiac failure and other fatalities, sudden cardiac death horses had fewer lifetime starts and were more likely to have died in training than in racing.[4] The objective of this study was to determine if racehorse exercise histories differed between racehorses that died from sudden death and matched control racehorses that did not die.

Lifetime past performance records of races and timed workouts (InCompass Solutions) were obtained for Thoroughbred racehorses that suffered sudden death (cases) between 2007-2020 (n=105) and 3 horses that did not die (controls) matched on last event (race or location and date of timed work, respectively), breed, sex, and age (n=315). Past performances were reduced to exercise variables consisting of counts of high-speed events (races, official timed works), lay-up (period of >60 days without a race or timed work), distances, and rates of events and distances during career and non-layup (active) portion of career using a custom Matlab program as previously reported.[5] Exercise variables were compared between cases and controls using a one-way ANOVA, Wilcoxon test, and using conditional logistic regression (SAS 9.4, SAS Institute Inc.). Continuous candidate risk factors were examined for linearity of the odds using the Box–Tidwell transformation. Categorical transformations were examined for continuous variables with non-linearity of the odds by examining data ranked by quartiles and ranks determined by observed cutpoints. Variables with p values ≤0.05 were considered statistically significant. Multivariable logistic regression models used stepwise and backward processes using the AIC criterion for model comparisons. A univariable p value <0.20 was used for initial selection of candidate variables for multivariable models. Horse age (days) and sex (female, male) were included in candidate variables. Final model fit was examined using the Hosmer and Lemeshow Goodness-of-Fit test and area under the receiver operating curve.

Compared to their exercise event matched cohort, sudden death horses were on average 3 months older (105 days), had longer careers (97 days), slightly longer furlongs per work and per event (0.1 furlong per work or event), more lay-up (74 days), and shorter distance in last month (1.9 furlongs), and were backing off distance in the last month (2.9 furlongs) ($0.001 \geq p \leq 0.040$). Multivariable conditional logistic regression without consideration of age and career length variables found that the odds ratio for a difference in mean values between case and control was 5.326 for 0.1 furlongs/work ($p<0.001$), 0.00168 for 74 lay-up days ($p=0.004$), and 0.913 for 1.9 furlongs in the last month ($p<0.001$).

These findings indicate that sudden death horses may show prodromal signs of a problem before death that trainers respond to by backing off training.

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Stephen Smith founded sports technology company Kitman Labs in 2012 to help professional and elite sports teams leverage data and analytics to unlock human performance. Prior to founding Kitman Labs, Stephen was Senior Injury Rehabilitation and Conditioning Coach with the Leinster Rugby Club in Ireland for six years. Stephen earned his undergraduate degree in Sport and Exercise Rehabilitation from Carlow Institute of Technology, and his master's degree in Football Rehabilitation from Edgehill University in 2011. Stephen's master's thesis investigated the analysis of combined risk factors as predictors of athletic injury; this research served as the foundation for Kitman Labs.

Kitman Labs is now the world's leading sports science and performance intelligence company with an established track record of working with more than 2000 top governing bodies, leagues, and teams in 26 countries, including some of the world's most elite sports organisations and universities such as the NFL, NBA, Premier League, MLS, NWSL, and NCAA. Kitman Labs powers iP: The Intelligence Platform (iP), the advanced operating system designed to optimise human performance; enhance overall health, wellness and longevity; reduce injury risk; and drive operational efficiencies and value. iP currently represents the industry's largest network of elite and youth teams and the industry's largest talent, performance, and medical dataset for all stages of the athlete lifecycle. Kitman Labs is headquartered in Silicon Valley with offices in Dublin.

8

APPLICATION OF WEARABLE TECHNOLOGY TO ACCURATELY QUANTIFY VARIABLES ASSOCIATED WITH PERFORMANCE IN RACING THOROUGHBRED HORSES

Warwick Bayly[1], James Meyer[2], Kevin Donohue[3], David Lambert[3]

[1]Dept Vet Clinical Sci, Washington State University, Pullman, WA USA; [2]206 Bossard Road, Groton, New York 13073-9778; [3]StrideSAFE USA, 107 W. Main Street, Midway, KY 40347

While, to many, the ultimate measure of performance is an individual's finishing place, the true assessment of performance in most human running athletic activities involves quantifying of how fast they run. Major advances in wearable technologies over the last 30 years have greatly enhanced the number and accuracy of performance-related variables that can be measured in most human athletic endeavors. However, as yet, the North American thoroughbred (TB) racing industry has been slow to adapt these technologies to the assessment of racehorse performance. For example, the finishing time assigned to a horse and associated calculations of its average speed are invariably inaccurate because: i) there a is highly variable distance (called the "run up") between the starting gate and the point where the race and its timing officially start. This distance varies widely with the racecourse, the track surface and the race distance; ii) the "winning" time is actually the time that elapses from the moment the first horse completes the run up until the race winner crosses the line. Often, these are not the same horses; iii) the official race distance is measured along at the rail from the end of the run up to the winning post. Therefore, every horse runs further than the published race distance and, according to how the race is run, some horses run much further than others; and iv) the times assigned to the non-winners are likely to be even more erroneous because their distance behind the winner is eyeballed based on their track position when the winning horse cuts the timing beam. Although the rest of the field has yet to finish, their eyeballed distance behind is converted to seconds (usually 5 lengths = 1 sec) regardless of how fast individual horses are going, and that is their assigned time. Under these circumstances, attempts to calculate standard measures like average speed are virtually meaningless. Given the increased public concern regarding the purported use of performance enhancing drugs in TB racing, the absence of a means to accurately quantify TB performance makes it extremely difficult to determine if a particular agent does or does not enhance a horse's racing performance. We investigated whether existing wearable technology could be adapted to more accurately measure performance in TB racehorse.

The study had 4 specific aims. They were to: i) evaluate the differences between track locations in actual distances run and average speeds on dirt and turf tracks under equivalent track surface conditions; ii) compare the distances run and average speeds for dirt and turf surfaces under equivalent surface conditions over the same official distances at a specific track location; iii) determine within-horse variability in average speed when the same horses race the same distance on the same track under the same track condition; and iv) assess how track surface affects average speed of individual horses racing over the same distance at the same track location. Data were analyzed with the Kruskal-Wallis non-parametric test ($p<.05$).

4,282 horses aged ≥3 years started 10,501 times in 5,884 1200m races (dirt-5404, turf-480) and 4,707 1600m races (dirt-3158, turf-1549) over 20 tracks located at 8 racecourses between July 2021 and November 2024 while wearing global positioning sensors (GPS) that were accurate to 0.01 sec and 0.001% for distance. Except for one track (#14), there were no between track differences in the distances horses ran on dirt surfaces. Horses racing on turf ran further than did those running the same official distance on dirt, particularly when at the same location. Horses racing on turf had significantly faster average speeds than those running on dirt over the same race distances (Table 1). This finding was reinforced when average speeds of horses (n = 113) that ran over the same distance on both dirt and turf at the same location under equivalent surface conditions were compared. When they ran over turf, the average speed of these horses was faster by 0.56 (95%CI: 0.46, 0.66) m/s; $p <0.001$; Figure 1).

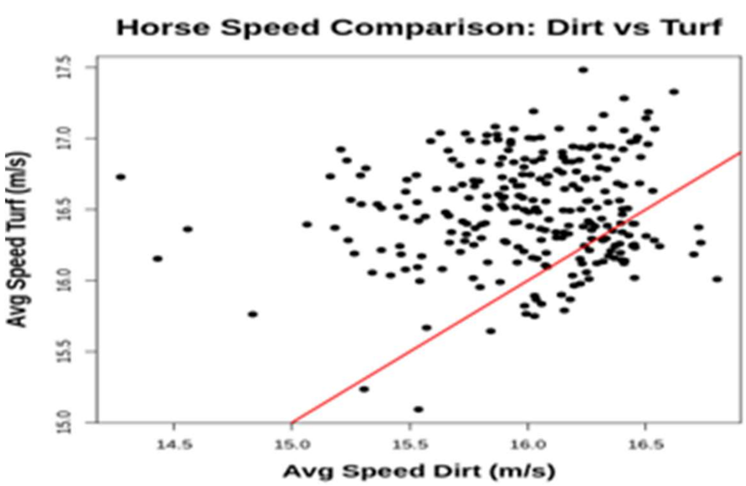
Within-horse variability in average speed was determined by calculating the mean of the standard deviations of each horse's average speed over the given race distance on the same racetrack surface and condition. There were ≥20 horses in each category and every horse had ≥2 races. Only 4 tracks had sufficient horses to meet these criteria, and all but one group of races were run on dirt surfaces (Table 2). The results indicated that within-horse variability was extremely consistent at a given track and was similar over both 1200 m and 1600 m on dirt tracks even though different horses contributed to each dataset. Whether the same finding applied to turf surfaces could not be ascertained because of insufficient data points.

In conclusion, the use of GPS sensors provided a robust and repeatable method for accurately quantifying time, average speed, distances run, and performance variability in Thoroughbred horseracing. Unlike traditional metrics such as finishing position and estimated race time, which can be influenced by numerous external factors, average speed offers a more precise indication of a horse's performance. Within-horse variability in average speed is particularly valuable as it establishes a baseline for how much a horse's performance typically fluctuates under normal conditions. This baseline is crucial to the evaluation of the impacts of interventions such as medications or training changes on performance, as it allows for more objective assessments of whether observed improvements are beyond the range of normal variability. Wearable technology has the potential to transform performance assessment in horseracing. For example, in addition to the parameters above, it could measure and compare variables like rate of acceleration and times over the first 40 m, and assess how race distance, age, track surface, track condition, and course layout influence these measures. The adoption of such advanced monitoring tools by horseracing authorities would align the sport with contemporary practices in human athletics, paving the way for enhanced performance analysis and improved welfare for equine athletes.

Table 1. Mean (95% CI) within-horse variability in average speed in races over 1200m and 1600 m based on races in which ≥20 horses raced >1 time over the same distance on the same surface and track condition at the same location.

Track ID	**Distance**	**Surface**	**av SD m/s**	**horses**
4	1200 m	Dirt	.27 (.24, .30)	195
4	1600 m	Dirt	.27 (.25, .30)	188
14	1200 m	Dirt	.16 (.15, .18)	237
14	1600 m	Dirt	.18 (.14, .22)	69
14	1600 m	Turf	.15 (.10, .23)	20
24	1200 m	Dirt	.13 (.10, .16)	20
24	1600 m	Dirt	.16 (.10, .22)	34
25	1200 m	Dirt	.18 (.15, .19)	84

Figure 1. Average speeds of horses that raced on dirt and turf over the same distances and surface condition at the same location. Horses were significantly faster over the turf ($p < 0.0001$).



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VALIDATION OF A NOVEL WEARABLE SENSOR TECHNOLOGY IN EQUINE GAIT ANALYSIS.

Sandro Colla, Melissa King

Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO USA

In sporting scenarios, the constant pursuit of performance improvement is closely correlated to injuries. This is particularly relevant in equestrian sports due to the high rate of injuries and increasing concern from the general population related to the social license for using animals in sports and the overall welfare of equine athletes. Thus, a better understanding of equine biomechanics in lame and non-lame horses is critical. Historically, biomechanical studies frequently involved optical motion capture systems (OMC) and force platforms, and they remain the gold standard for kinematic and kinetic analysis, respectively. Both systems provide validated objective temporal, spatial and force data, establishing the foundation for understanding equine biomechanics. However, they have several limitations, such as being confined to a laboratory environment, frequently limited to straight lines and small circles during walking and trotting, and reduced number of consecutive strides analyzed per trial. The use of wearable inertial measurement unit sensors for the collection of equine biomechanical data is increasing in popularity, in part due to the ability to capture movement during sports-specific activities outside of traditional biomechanics laboratories. Inertial measurement unit systems are sensors able to collect spatiotemporal (kinematics) parameters, such as acceleration, orientation, angular rates, and gravitational forces. These lightweight sensors can be attached to different body segments, collect real-time data, and transmit it wirelessly to a mobile phone application during different activities. A novel body-mounted IMU sensor system integrated with machine learning and computational algorithms has been validated with optical motion capture systems and force platforms in different human subjects performing several functional tests and sport-related activities. The ability of this novel system to provide both kinematic and especially kinetic parameters in real-time has allowed coaches and trainers to monitor individualized training programs, improving performance and reducing injury risk. In horses, various body-mounted IMU systems have been validated in sound and lame horses under different conditions and were able to provide spatiotemporal parameters, but biomechanical loading using IMU systems has not been previously described. This novel technology's promising capability to provide real-time kinetic and kinematic data in horses will complement our understanding of equine biomechanics, promoting individualized training, enhancing performance, and mitigating injury risk. Our overall goal is to critically evaluate the use of the proposed novel IMU sensor technology to quantify biomechanical loading characteristics using an equine reversible lameness model. We hypothesize that application of novel inertial sensor technology and machine learning techniques will provide data as accurate and reliable as those reported from force plates and optical motion capture systems. This hypothesis has led to the development of our primary specific aim to determine the reliability and accuracy of IMU based metrics to calculate biomechanical loads using an equine reversible lameness model.

Eight mixed-breed horses were utilized in a cross-over design study with weekly data collection at baseline and with induced 1/5 (mild), 2/5 (moderate), and 3/5 (marked) AAEP scale forelimb lameness. Lameness was induced in one randomly selected forelimb with a modified horseshoe containing a frog plate and a guide hole for a single set screw with a hex driver head that can be tightened into the corium of the frog. The varying degrees of reversible supporting forelimb lameness were randomly induced weekly in each horse. Twelve wireless nine-axis (triaxial accelerometers, gyroscopes, and magnetometers) IMU sensors, developed by Vayu technology were rigidly attached with Velcro to the poll, bilaterally over the lateral aspect of the mid metacarpus and metatarsus, over the sternum, bilaterally over the lateral aspect of the mid radius and tibia and over both tuber coxae. IMU, OMC, and force platform data were collected simultaneously during five trot trials for each horse under each lameness scenario. Bland-Altman and intra-class correlation coefficients will be utilized for statistical analysis.

Preliminary results from baseline data demonstrated an average peak error of 3.26 ± 2.29% (left forelimb) and 2.81 ± 2.02% (right forelimb) in the vertical ground reaction forces (Figure 1) and 0.011 ± 0.009 seconds (left forelimb) and 0.011 ± 0.008 seconds (right forelimb) in the stance time between the data obtained from the IMU sensor-based system compared to those from the force platforms. Utilized as a critical metric for evaluating the accuracy of methods used to estimate vertical ground reaction forces using IMUs and machine learning algorithms, the peak error was calculated as the difference between measured peak vertical ground reaction forces (PFz) and predicted peak ground reaction forces divided by the measured PFz multiplied by 100 ((PFz-predicted IMU peak GRF)/(PFz))*100. The calculated low percent errors are promising for the initial baseline data reported here and warrant further data analysis and additional statistical methods to determine correlation coefficients between the data sets. The ability to quantify biomechanical loading in real time under field sport-specific activity provides untapped information into whole body loads during training and exercise. Further studies are encouraged to test the use of this novel technology during sport-specific activities and rehabilitation programs.

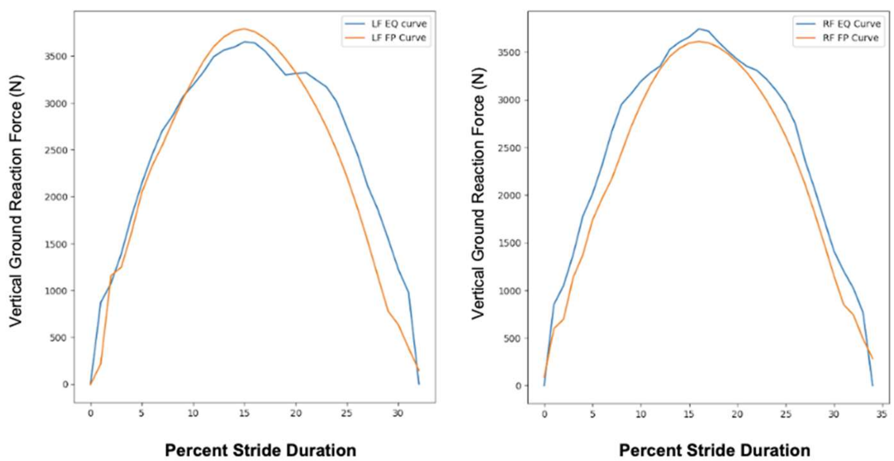

Figure 1: Vertical ground reaction force-%stride duration graphic. Left front limb (left) and right front limb (right). Solid blue line (IMU system), Solid orange line (force platform).

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LESION SPECIFIC ALGORITHMS CREATED FROM ACCELEROMETER DATA CAN PREDICT CONDYLAR AND SESAMOID FRACTURES IN RACEHORSES

David H. Lambert[1], Mikael Holmstrom[1], Kevin Donohue[1], Denise Mc Sweeney[2], Warwick Bayly[2]

[1]StrideSAFE USA, 107 W. Main Street, Midway, KY 40347; [2]Department of Veterinary Clinical Sciences, Washington State University, Pullman, WA USA

The purpose of the study was twofold. Firstly, to create targeted algorithms from racing accelerometer data with the aid of artificial intelligence (AI) techniques to identify thoroughbred racehorses most at risk of suffering a future front limb condylar or sesamoid fracture. And secondly, to explore the feasibility that, by using these algorithms throughout a racing career, horses at high risk for these injuries which could be identified and sent for a positron emission tomography (PET) scan for further evaluation of their metacarpal condyles or sesamoids and avoid such an injury rather than incurring it.

Data was collected during races using an 800Hz, 3-axis accelerometer system coupled with a 5Hz Global Positioning System (GPS) and then downloaded for analysis and secure storage. Before every race each horse was allocated a sensor programed with its Jockey Club identification which was then placed in a pocket in the saddle cloth positioned over the lumbar vertebrae. Activation was by GPS 5 minutes before post time but could be overridden if the official post time changed. Data was collected from the starting gate to the finish. The accelerometers provided 800 measures/sec of the acceleration forces experienced by the racing horse in the dorsoventral, longitudinal, and mediolateral orthogonal planes. This equated to ≈1000 data points/stride from which 194 variables describing every stride had been identified and an overall risk screening algorithm built. Data files were generated from 36,597 race starts which included 47 races by 26 different horses that eventually suffered a condylar fracture, and 77 races by 35 different horses which sustained sesamoid fractures. These files included the race in which the fracture occurred. Diagnoses were confirmed at necropsy or post-race veterinary examination. The 2 front limb condylar- and sesamoid-specific algorithms were created from this dataset of race starts.

Creating viable algorithms required their initial development followed by a respective validation phase. Prior to the development phase, 20% of condylar fracture runs and 20% of the sesamoid fracture runs were randomly removed from the pool of files for horses that sustained fractures, for later use in validating the algorithm.

A Linear Model Builder (Statistica 12, StatSoft) was used to identify which of the 194 variables showed statistically significant differences between condylar fracture runs, sesamoid fracture runs, and the remainder of the runs. Wherever possible, data from runs up to 12 months before the horse's final race was used. As 80-90% of catastrophic injuries occur at sites of pre-existing pathology, it was reasoned that early pathology would have a negative impact on stride characteristics and recognizing such patterns could provide an early opportunity to intervene, treat the horse, and so prevent a fracture. For developing these fracture algorithm models, stride dynamic variables which were used included peak and average accelerations within each stride, timing for critical changes in each phase of the stride, and the distribution and timing of forces between the 3 orthogonal planes. Data from both the back straight of the racetrack and the final or only turn were collected and compared. To complete the modelling, AI tools were applied with the goal being to create specific algorithms to accurately identify those runs which indicated that a horse might be at increased risk of either type of forelimb fracture. Four levels of risk were identifiable as was any asymmetry in the stride. This produced 5 risk of injury categories as follows.

Category 1 had 22,118 runs which included 15 fracture runs (0.07%). Category 2 had 7,562 runs with 6 fracture runs (0.08%). Because of the similarity in fracture incidence, these 2 categories were assigned a risk factor =1. Category 3 had 4,864 runs with 26 fracture runs (0.5%) indicating such horses were at 8 times greater risk than the Category 1 or 2 horses of incurring one of these fractures. Category 4 had 705 runs with 17 fracture runs (2.4%); ie, a 35-fold greater risk. Category 5 had 709 runs with 60 fracture runs (8.7%) reflecting 125 times higher risk than horses in Category 1 or 2. Mediolateral asymmetry during the front leg stance phase of the stride helped identify which front leg was involved. Increased G forces to the left usually resulted from a horse avoiding or "getting off" its right front and vice-versa. The post-race veterinary report based on these algorithms could therefore include a risk category rating, the limb believed to be involved, and which of the two fracture types was most likely.

In order to further investigate the usefulness of the algorithms that were developed and investigate the feasibility of identifying horses most likely to suffer either fracture in order to facilitate clinical intervention before they broke down, all the runs for horses with either fracture type were compared to all the runs from 100 “control” horses which had received at least one Category 3-5 rating in the previous 12 months but had not suffered one of these fractures. To make this comparison, a sliding risk category average was calculated using a fixed subset of 3 runs. After 3 races, the category ratings for each were averaged. For example, a 2-3-4 gave an average of 3. If the horse’s next race received a Category 1 rating, then the calculation became 3-4-1 for an average of 2.7. Six of the horses with condylar fractures had 10 sliding average calculations, and 10 horses with sesamoid fractures had 17 such calculations. The control group of 100 horses had 524 calculations.

After running the condylar algorithm against the population of 36,597 starts, 4,194 runs were given a Category 3-5 rating and this group contained 43 of the available 47 fracture runs (1.025%; i.e., there were 91% of the fracture runs in 12% of the races). This algorithm then underwent validation testing using the 20% of fracture runs excluded from its development, and 8 out of 9 (89%) of the runs in which condylar fractures occurred were correctly identified. When applied to all the data files, the condylar algorithm also identified 31,794 Category 1 runs which contained just 4 fracture runs (0.013% compared to the 1.025% for Categories 3-5) meaning that when the condylar algorithm was applied to the total population of race starts, those horses given a Category 3-5 rating were at 80 times greater risk of a condylar fracture than those given a Category 1 rating.

Running the sesamoid algorithm against the total population of race starts resulted in 5,165 runs receiving a Category 3-5 rating. This group contained 60 of the 77 available fracture runs (ie, there were 78% of the fracture runs in 14% of the total races for an overall incidence of 1.1616%. The sesamoid algorithm was also then validated against the 20% of fracture runs excluded from the algorithm’s development 14 of the 15 (93%) of fracture runs were correctly identified. The sesamoid algorithm also classified 30,792 races as Category 1 runs. These contained 17 fracture runs (0.0552%), indicating that when the sesamoid algorithm was run, horses given a Category 3-5 rating were at 20 times greater risk of a sesamoid fracture than those given a Category 1 rating.

To judge the usefulness of the sliding category average protocol, a sliding average threshold of 2.5 was used to compare Category 3-5 populations that did and did not suffer condylar or sesamoid fractures. For horses that suffered condylar fractures, all 10 sliding average calculations were above the 2.5 threshold. For horses with sesamoid fractures, 15 of 17 calculations were above the 2.5 threshold. (A single horse had 2 calculations below the threshold, but the same horse also had a third run which was above the threshold.) In the case of the 100 Category 3-5 “controls”, 23 horses (23%) had at least 1 calculation above the threshold. Therefore, 77% of the horses which were rated Category 3-5 but which did not succumb to either of these two types of fracture could be separated from the population which died. The incidence of categories 3-5 identified by the overall risk algorithm was 17% of the 36,597 race starts. This 17% included 91% of the condylar fracture horses and 82% of the sesamoid fracture horses. Factoring this overall 17% with the 23% of Category 3-5 horses with a sliding category average >2.5, means that only 4% (0.17 x 0.23 = 0.04) of the total population of race starts contained 91% of the condylar fracture horses and 82% of the sesamoid fracture horses.

The algorithms we created from the accelerometer sensor data were able to distinguish between races by horses at high risk of suffering a front limb fetlock fracture of its sesamoids or a metacarpal condyle, and horses at little or no risk of incurring them. After 3 runs, the use of the sliding category average calculation the list of high-risk horses could be found in just 4% of the 36,597 race starts. A veterinarian familiar with sensor technology and the sliding category average calculation could have weeks or even months in which to diagnose the fetlock problem before it fractures and so protect and save the horse. Horses with a Category 3-5 rating and a sliding average of greater than 2.5, if not passed as healthy by the attending veterinarian, would be suitable candidates for a PET scan. As the accelerometer system also identifies mediolateral asymmetry, then the effected limb could also be identified. Over time, a consistent application of this methodology at a racetrack could identify and remove from high-speed work, more and more of those horses with serious fetlock disease as confirmed by PET scan. If the veterinary profession applies all these tools to the care of racehorses, it is not unreasonable to predict that eventually condylar and sesamoid fractures could be largely prevented in thoroughbred horses racing in America.

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HOOF-ON AND -OFF DETECTION DURING GALLOP USING A SINGLE INERTIAL MEASUREMENT UNIT

Maialen Matray[1], Camille Hebert[1], Guillaume Dubois[1]

[1]*LIM France, 8 avenue de l'hippodrome, 33110 Le Bouscat, France*

Musculoskeletal injuries (MSI) are the most common cause of death in thoroughbred galloping horses, accounting for about 70% of race-day fatal events. It is thus of crucial importance to detect these injuries in the earliest stage. Over the last decades, numerous epidemiological studies have attempted to identify risk factors for MSI, providing low-risk training strategies to reduce their occurrence and impact. Hoof-on and hoof-off relative timings are essential locomotion parameters for horse lameness detection. Musculoskeletal pain induces compensatory mechanisms that may impact the horse gait pattern [1]. At trot, horses with moderate lameness decrease their relative stance duration by 1.1% for forelimbs and 0.7% for hindlimbs. According to Peham et al. [1], standard deviation of stride length can also increase by up to 0.35% in horses with a visible forelimb lameness. Although the effect of musculoskeletal pain on gallop patterns remains to be investigated, similar compensatory behaviors can reasonably be expected. In this regard, locomotion events detection seems to be a valuable tool to discriminate at-risk galloping racehorses. Inertial Measurement Units (IMUs) offer promising new prospects for in-field locomotion analysis. However, the use of four limb-mounted sensors is inadequate for daily use on racehorses because of added distal limb weight, inconvenience of use, and safety matters. In-field analysis of racehorse locomotion should ideally be performed without instrumenting the horses' limbs. Such issues have already been addressed in human biomechanics, using deep learning models. To the authors' knowledge, the detection of all hooves' contacts of galloping thoroughbred horses has never been achieved using a single IMU. In that context, the present study introduces and validates a deep-learning algorithm for hoof-on and -off instants detection during gallop using a single IMU attached to the saddle girth.

Data were recorded using five IMUs (Blue Trident, Vicon, the Netherlands): one on each limb, securely attached on the boots and one taped on the saddle girth. All IMUs were synchronized with one another using the app provided by the manufacturer. Sampling frequency was 1600Hz and 1125Hz for accelerometer and gyroscope respectively, and IMUs had a full-scale range of 200g and 2000°/s. Acquisitions were carried out during 34 daily trainings of 32 different thoroughbreds, aged from 2 to 7 years. The final dataset included 20008 strides of gallop with speed ranged from $3.9 m.s^{-1}$ to $16.7 m.s^{-1}$.

First, hoof-on and hoof-off instants from limb IMU's data were detected based on a method derived from an algorithm at walk and trot [3], and validated on 10 horses with IMU on hooves. It provided reference events that were used to create 4 ground truth binary signals, one for each limb of the horse. A value of 1 was assigned for the stance phase (foot in contact with the ground) and 0 for the swing phase (foot off the ground).

Then, one supervised machine learning models per limb, CNN-LSTM Encoder-Decoder, were trained to predict binary signals instants from the solely saddle girth IMU's data. Input data consisted of the saddle girth IMU's acceleration and angular velocity signals along x, y and z, their Euclidean norm. Input data were normalized and to avoid subsequent errors in prediction due to differences in horse's morphology and sensor's position and prevent over-fitting, each sample acceleration and angular velocity data were multiplied by a rotation matrix derived from an arbitrary rotation axis and angle.

The sampling frequency was 200Hz, signals were divided into samples of 256 timesteps (1280ms) using a sliding window with a step of 10 milliseconds. Data from 20 horses were used to train the Encoder-Decoder and 7 were kept for validation. Data from 7 remaining horses were used to evaluate the developed method compared to the reference method. Training, Validation, and Test datasets were then switched twice so that the method was eventually tested on 21 different horses.

The predicted hoof-On and hoof-Off events derived from the decoder output were compared to the reference events. The average and standard deviation of the time differences between the predicted and reference values were presented both in milliseconds and as percentages of the total stride duration in table 1.

Table 1 Mean (STD) of the difference between predicted and reference hoof-on and hoof-off events in milliseconds and in percentage of the stride duration

	Hoof on		**Hoof off**	
	ms	**stride %**	**ms**	**stride %**
Lead Forelimb	3.9 (13.0)	0.73 (2.49)	2.6 (15.3)	0.49 (2.83)
Trail Forelimb	-0.6 (14.6)	-0.09 (2.8)	2.5(15.5)	0.46 (2.92)
Lead Hindlimb	-2.7 (18.0)	-0.50 (3.37)	-1.3 (15.4)	-0.25 (2.95)
Trail Hindlimb	0.3 (16.6)	-0.09 (3.17)	2.4 (14.3)	0.44 (2.73)

The model performed equivalently on all limbs. Bias was lower than 0.73% of stride duration for foot-on for both forelimbs and hindlimbs, and 0.49% of stride duration for heel-off. The method thus provided similar accuracy than previous IMUs based detection methods, while requiring only one IMU. The whole process was carried out at a sampling frequency of 200Hz, meaning there are 5 milliseconds between two frames. Thus, this protocol achieved a bias lower than 0.8 (std 3.6) frames for foot-on and 0.5 (std 3.1) frames for heel-off. Increasing sampling rate might reduce the bias of the order of magnitude of the sampling period; but would also require greater computational power to train the machine learning model. The dispersion may also result from an overstated dispersion of the reference events. For an improved accuracy, the detection algorithm should be compared to gold standards, such as force places or motion capture-based algorithms. Nonetheless, these technologies would be difficult to implement with galloping racehorses as they narrow the area of data collection to pathways a few meters long and not compatible with training environment.

Our results show a high level of agreement for stride duration ranging between 425 and 500ms with Witte et al.'s results [4]. However, they did not reveal any significant difference between lead and trail limbs' duty factors, our results indicated that a significant difference may be found for higher stride duration.

Previous publications tried to quantify locomotion changes at trot, during artificially induced lameness. A mild or moderate lameness results in a significant decrease in stride duration (11ms/31ms for mild/moderate lameness) and suspension duration (13ms/33ms), and in a significant increase in relative stance duration (1.1% / 3.0% for hindlimbs and 0.7% / 1.8% for hindlimbs). Also, visually obvious lameness can result in mean stride duration decrease of up to 30ms, and a mean percentage stance duration increase of up to 2.1%. The authors could not find any study relating to MSI-induced gallop pattern modifications. Further studies are needed to state to what extent this method can help detect MSI. It may not be accurate enough to detect subtle anomalies in gallop locomotion yet, however it proved sufficient accuracy to detect moderate lameness.

By overcoming the necessity to equip the horses' limbs, this study opens the way to gait events detection in use-cases that have hardly ever been investigated today. For now, is has only been validated on galloping racehorses, yet the method could easily be adapted to trotting racehorses or sport horses. The great simplicity of the instrumentation required by our algorithm will undoubtedly enable in-depth analysis of the locomotion of galloping racehorses directly on the training ground.

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COMPARISON OF THREE OBJECTIVE MEASUREMENT SYSTEMS AND TWO SUBJECTIVE EVALUATORS IN NATYRALLY OCCURRING EQUINE LAMENESS

Jenna L. McPeek, Bruno Menarim, Beatrice Sponseller, Margaret McClendon, Amanda A. Adams, Allen E. Page

Department of Veterinary Science, Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY USA

Lameness evaluations are an intrinsic component of equine musculoskeletal health assessment in both clinical and research contexts and are predominantly performed in a subjective manner. Such subjectivity presents a significant challenge for agreement between multiple evaluators, while introducing observer bias and providing data that is only qualitative in nature. While typically sufficient for in-field diagnostics and veterinary care, scientific studies often require more precise and repeatable techniques. In recent years, several methods to assess equine gait asymmetries have become available. These systems aim to identify affected limbs and quantify the degree to which each limb is affected.

This study sought to evaluate the agreement between two experienced veterinary evaluators and three commercially available, objective measurement systems (an artificial-intelligence-based smart phone application (Sleip) and two inertial sensor systems (Equinosis Q, 3 sensors; Equisym, 7 sensors)). Eighteen research horses (15 – 30 years old) with varying degrees of naturally occurring lameness were assessed. Each horse was jogged in hand by one of two experienced handlers in a straight line on a 30-meter asphalt path six times (three round trips). For the inertial systems, all sensors were placed using manufacturer attachments and instructions. The head and pelvis sensors shared locations and were placed immediately in-line with one another. Data output from each observer or system was converted to an ordinal scale (0 to 3) based on recorded degree of asymmetry. A score of 0 indicated no asymmetry or a very mild asymmetry while a score of 3 indicated a prominent or severe asymmetry. Each limb was scored independently. Veterinarians were allowed to observe horses both straight on and from the side while the Sleip app recording was only obtained straight in front of or behind the trotting path. While default analysis settings/algorithms were used with all objective systems to prevent manipulation of results, the lack of automated interpretation from the Equisym system required manual analysis of the output data to assign ordinal values. Based on discussions with the company and their experience with the system, Equisym amplitude asymmetry values were defined as none/very mild (0-27%), mild (28-39%), moderate (40-51%), and prominent (52%+) for the head sensor and as none/very mild (0-16%), mild (17-24%), moderate (25-32%), and prominent (33%+) for the pelvis sensor. The Equisym withers sensor was not used for data analysis in this study. Agreement in asymmetry scores were quantified via weighted Cohen's Kappa and *P*-values were less than 0.05 unless noted.

An averaged weighted Cohen's Kappa score for all raters and limbs was determined to be k = 0.56, indicating a moderate level of agreement. Objective evaluator agreement (k = 0.70) was higher than subjective evaluator agreement (k = 0.41) across all limbs. Overall agreement for forelimb asymmetries was greater (k = 0.63) than overall agreement for hind-end asymmetries (k = 0.44). However, objective systems had a higher agreement with hind limb asymmetries (k = 0.61) when compared to the subjective evaluators (k = 0.15, $P>0.05$). A similar pattern was present between objective (k = 0.81) and subjective evaluators (k = 0.67) for forelimb asymmetries, although the difference in agreement was not nearly as pronounced. Pairwise comparisons of weighted Cohen's Kappa scores are presented in Table 1 for the various subjective evaluators and objective systems. Individual agreement scores between the objective systems were generally less variable and typically higher than the veterinary evaluators, with the exception of Vet #2 and the evaluation of forelimb asymmetry.

Data from this project suggest that objective systems are less apt to variability when detecting subtleties in hind limb asymmetries compared to straight-line veterinary examination. Further, the objective systems had consistently higher agreement, suggesting that there are only minor differences between the utility of each system. Additionally, based on individual cases evaluated in this study, it appears that the Equinosis Q and Equisym systems have some capacity to detect bilateral asymmetries on a straight trot, although these conclusions may require manual interpretation of the results. Simultaneously comparing multiple objective systems highlighted their differences in data output and user interface accessibility. The Equinosis Q system provides a significant amount of raw data, which could make it harder to quickly interpret but potentially be more useful in research situations. In contrast, the Sleip app has a user-friendly interpretation screen but does not provide raw data to explain the algorithm's findings. Finally, the Equisym system has an aesthetically pleasing and useful user interface but did not provide automated data interpretation at the time of testing. Limitations of this study included only jogging horses in a straight line as lunging and flexion tests would likely have affected the agreement between subjective and objective evaluators, as well as the ability to detect bilateral lameness. Additionally, repeatability of the findings for each objective system were not examined in this project. Finally, results were not compared against motion capture analysis, largely regarded as the gold-standard for asymmetry detection and objective system evaluation.

This project is the first to compare the Equisym system versus other objective measurement systems in combination with veterinary evaluation. Ultimately, the data presented here demonstrates that objective systems have a higher degree of asymmetry detection capabilities and agreement compared to veterinary examination when horses are trotting in a straight line.

*Table 2. Weighted Cohen's kappa, calculated for all limbs, forelimbs only, and hind limbs only between individual evaluators. P-values are <0.05 unless noted with *.*

All Limbs	**Vet #1**	**Vet #2**	**Sleip**	**Equinosis Q**
Vet #2	0.41	x	x	x
Sleip	0.58	0.59	x	x
Equinosis	0.39	0.51	0.73	x
Equisym	0.44	0.60	0.70	0.68
Forelimbs	**Vet #1**	**Vet #2**	**Sleip**	**Equinosis Q**
Vet #2	0.67	x	x	x
Sleip	0.54	0.93	x	x
Equinosis	0.42	0.72	0.82	x
Equisym	0.55	0.86	0.83	0.77
Hind Limbs	**Vet #1**	**Vet #2**	**Sleip**	**Equinosis Q**
Vet #2	0.15*	x	x	x
Sleip	0.65	0.28*	x	x
Equinosis	0.42	0.34	0.66	x
Equisym	0.33	0.40	0.58	0.60

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NOVEL METHOD FOR ASSESSING DRESSAGE HORSE TRAINING LEVEL USING GAIT PARAMETERS: A PILOT STUDY

Lise C. Berg, DVM PhD. Department of Clinical Veterinary Sciences, University of Copenhagen, Taastrup, Denmark. *lcb@sund.ku.dk*
Caroline Lynderup Hansen, DVM. Department of Clinical Veterinary Sciences, University of Copenhagen, Taastrup, Denmark
Laura Bue Koefoed, DVM. Department of Clinical Veterinary Sciences, University of Copenhagen, Taastrup, Denmark
Filipe M. Serra Braganca, DVM PhD. Department of Clinical Science, Equine Science, Utrecht University, Utrecht, Netherlands / Sleip AI, Stockholm, Sweden

Training-induced injuries are common yet preventable, making them an important target in our efforts to ensure horse welfare in sport horses. Exercise tests can be used to evaluate training status, optimise training programmes, and ensure timely intervention. However, most exercise tests are designed to evaluate aerobic capacity. Dressage horses rarely exceed their anaerobic threshold, and we therefore need a different approach for disciplines such as dressage, where cardiovascular capacity is not a useful indicator of training level.

If we observe dressage horses at different levels of training, it is apparent that the horses carry themselves differently and change their way of going as they advance through the levels. Studies have shown that gait parameters including stance and stride duration, stride frequency, protraction and retraction, diagonal advanced placement and vertical displacement are important for highly trained dressage horses (Back et al., 1994; Biau & Barrey, 2002; Holmström et al., 1994). We also know that balance, strength and stability are essential for horses to maintain healthy musculoskeletal loading patterns. Dressage training is often described on a 6-tier training scale, and progression through the training scale leads to a shift from a more pushing, more open movement to a more carrying, more collected movement as the horse gains more balance, strength, and stability (Reitvorschrift:(R.V.); H. Dv. 12, 1937). We propose that this progression affects the gait pattern quantifiably, and that gait parameters can be used as indicators of progression in training level.

The aim of this pilot study was to compare gait patterns between dressage horses at different training levels. We recruited 15 horses that were training and competing at low (N=5), medium (N=5), and high (N=5) level according to Danish Equestrian Federation competition classifications. All horses were reported to be in full work with no recent history of illness or lameness. Height, weight, body condition score, and muscle condition score were recorded. Seven inertial measurement units (IMUs, EquiMoves) were placed on the poll, withers, croup, and all four limbs. Previous studies have shown that IMU sensors can be used to measure gait and upper-body variables in sound horses (Bos, 2020; Bosch et al., 2018). The sensors provide data on stride, stance, and swing duration (time, ms.), protraction and retraction (degrees, °), adduction and abduction (degrees, °), and upper-body vertical movement and symmetry (distance, mm.). From these parameters, stride frequency, diagonal advanced displacement, and sagittal and coronal range of motion can be calculated. The horses were tested under two conditions: 1 – in-hand walk, slow trot, and fast trot on a straight line of approx. 25 m. on a firm surface with the handler alternating between sides of the horse at each speed, and 2 – on treadmill in walk at increasing speeds (increments of 0.5 km/h every 60 sec.) from almost too slow for the horse untill it struggled to keep up. Only horses familiar with work on a treadmill were included in part 2 (N=8). None of the horses were warmed up before the tests. Statistical data analysis was performed using Prism 9 (Version 9.5.0 (525)) for macOS. Investigators were blinded to horse training level during data analysis. Level of significance was set at $p<0.05$. One-way ANOVA was used in the in-hand test, and linear regression in the treadmill test.

The three groups of horses were similar in height, weight, and body condition score. Muscle condition score increased with increasing training level. The horses tolerated the study setup well. In the gait pattern analyses, we identified multiple parameters that were significantly different between groups. For the in-hand test, significant differences were found in front limb protraction in slow trot ($p=0.04$; 31.5±1.9 - 28.2±1.6 (°) and fast trot ($p=0.005$; 30.1±0.9 - 27.0±0.8 (°)), diagonal advanced placement in walk ($p=0.03$;

-29.6±3.5 - -22.1±3.7 (ms)), hind limb adduction in walk (p=0.01; -3.0±0.7 - -4.4±0.4 (°)), front limb abduction in slow trot (p=0.002; 3.5±0.8 - 6.1±0.7 (°)), hind limb abduction in slow trot (p=0.04; 5.2±0.6 - 6.9±0.9 (°)), and hind limb abduction in fast trot (p=0.007; 5.4±0.8 - 8.0±1.1 (°)). Three horses had to be excluded from the in-hand test analysis due to insufficient number of recorded strides. On the treadmill, significant differences were found in response to change in speed for stride duration (p=0.003; -0.13±0.02 - -0.03±0.02 (ms)), stance duration (p<0.0001; -0.12±0.01 - -0.04±0.02 (ms)), stride frequency (p=0.007; 0.07±0.01 - 0.02±0.01), and hind limb adduction (p=0.03; 0.3±0.1 - -0.3±0.2 (°)).

The findings of this study indicate that gait parameters may be a useful tool in evaluation and monitoring of training level and progression in dressage horses. Particularly, abduction and adduction limb angles appeared to be indicative of a more trained gait pattern. This makes biomechanical sense when considering the way quadrupeds balance. Ideally, limbs are not angled laterally or medially when loaded, since this creates uneven loading patterns on the musculoskeletal structures, but placing the limbs further from the center of the body creates a broader base and makes balancing easier. When the horse becomes stronger and more balanced, it has less need for this coping mechanism. Also, a more trained horse may have a more developed muscular thoracic sling, which will increase the space between the shoulders and thus the angle of the front limbs both in stance and locomotion. The limitations of this study include the small number of horses, but despite the resulting low power we were able to show differences between groups. We do not have the exact speed of the in-hand testing, but it is always a study design question whether standardisation of speed is appropriate, since different horses may prefer different speeds. However, a speed/response test would be interesting in order to determine, whether our findings are consistent when the same horse is tested at different speeds, and whether a slow jog or a more active trot is more precise. In our study, we decided not to test the horses when ridden. We wanted to avoid potential influence of the rider on the horse's way of moving, but future studies may include ridden tests. It will also be highly relevant to perform a longitudinal study to evaluate how sensitive the measurements are to day-to-day variations, and whether multiple measurements in the same horse over time can identify progression through the training scale.

In conclusion, despite the limited number of horses, several gait parameters showed a difference between training groups and may hold promise as indicators of training level and progression in dressage horses. More studies are needed to validate the results and determine the impact of gait pattern on dressage performance and injury risk.

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DAILY TRAINING WORKLOAD OF LOWER-LEVEL JUMPERS: PRELIMINARY RESULTS

Foreman JH, Foreman-Hesterberg CR, Foreman SE.

University of Illinois, College of Veterinary Medicine, 1008 West Hazelwood Drive, Urbana, IL 61802 USA

Real-time monitoring of physiological variables in exercising racing and event horses has been reported previously, but jumper training remains poorly studied. The objective of this study was to quantify the daily workload of lower-level jumpers in regular work. Daily exercise of horses in one jumper training stable were monitored using a HR/GPS monitoring system validated for use in exercising horses (ARIONEO EQUIMETRE, Paris, France). Horses were monitored in regular work during either individual training or small group lessons (2-to-5 horse/rider pairs/lesson). Variables monitored included total distance, duration, HR, stride length (SL), and velocity of work. Mean±SD results were calculated and were compared between subgroups where appropriate using the Student's t test with significance set at $P<0.05$. Twelve horses (mean age 15.6±6.0 years old; WB 7, TB 3, QH 2; 9 geldings, 3 mares) were monitored at a walk, trot, canter, and jumping on 56 occasions. Mean outdoor ambient temperature was 20.3±6.4 C (range 4.1-31.5 C) with no rain. Mean exercise distance and duration were 2.9±0.8 miles (range 1.4-5.0 miles) and 49.8±17.9 min (range 24-88 min), respectively. Mean HR_{peak} was 160±20 beats/min (range 122-211 beats/min) at a mean canter V_{peak} of 248.2±28.3 m/min (range 190-330 m/min). Mean SL_{peak} was 9.3±1.2 ft/stride (range 7.0-12.3 ft/stride). Exercise distance (2.3±0.6 miles) and duration (35.0±9.2 min) were shorter in individual training sessions compared to small group lessons (3.3±0.7 miles and 62.6±12.9 min, respectively) ($P<0.001$). It was concluded that these jumpers averaged peak effort at 67% of potential maximum using a conventional HR_{max} of 240 beats/min.

15

HOLISTIC USE OF INERTIAL SENSORS FOR DETECTING IMPENDING INJURY IN THOROUGHBRED RACEHORSES IN AUSTRALIA

Ashleigh V. Morrice-West, Adelene S. M. Wong, R. Chris Whitton, Peta L. Hitchens

Equine Centre, Melbourne Veterinary School, University of Melbourne, Victoria, Australia

Musculoskeletal injuries in Thoroughbred racehorses constitute a major threat to horses, their riders, and the social licence of the racing industry. This has prompted considerable international research efforts in recent years investigating how and why such injuries occur, to ultimately reduce preventable incidents. Injuries in racehorses are not typically a spontaneous event, but rather develop gradually due to the cumulative effect of bone loading with each stride (bone material fatigue). Stride characteristics for individual racehorses can now be recorded with wearable technology and this information used to quantify the impact of galloping on the skeleton over time. We therefore used hybrid epidemiological, machine learning and mathematical approaches in a three-part study, to identify factors that predict raceday injuries. We also considered alternative adverse outcomes: enforced rest and retirement.

Race start data for Thoroughbred racehorses competing in Tasmania, Australia, between 10 July 2011 and 21 August 2016 were extracted from the official racing repository (Racing Australia Ltd.), including data related to the race, track, horse, and its previous racing history. Racehorse injury data was obtained from the Australian Racing Incident Database (ARID). For both datasets, historical data from 1 August 2004 were obtained. Records were classified as a musculoskeletal injury if reported in the free text or condition classification fields as being lame or showing gait abnormalities, shin soreness, fractures, bone injuries, tendon, or ligament injuries. Enforced rest was defined as a period of >22 weeks between races and/or trials. Horses were considered retired if their last career race was within the study period. Stride data were collected using StrideMaster™ devices mounted on saddle cloths of each horse for every race start. The devices contain GPS, Global Navigation Satellite System (GLONASS) and 3-axis accelerometers with 5 Hz positional and 800 Hz accelerometer data capture. Stride characteristics, including speed (m/s), stride length (m), and stride frequency (strides/s) were available as summarised values (means) per 200m ("sectionals"). Data were excluded if biologically implausible (speeds >21 m/s, stride lengths >9.2 m and stride durations <0.37 seconds). There were n = 153,315 sectionals in n = 25,259 race starts by 2,678 individual horses available for analysis. Findings are presented as Hazard Ratios (HR) with 95% confidence intervals (95% CI) at a significance level of $P < 0.05$.

The aim of Part [1] of the study was to determine whether changes in stride characteristics over each successive race start (unit of time) were associated with injury. In a case-control subset, we modelled the final 200 m of each race, as this final sectional was expected to represent each horse's peak exertional capacity. We used a multivariable approach that jointly modelled the (i) changes in stride characteristics over successive race starts; and (ii) the time to injury as a proportional hazard.

The aim of Part [2] was to determine stride characteristics that were predictive over different windows of time prior to an injury or alternate adverse event using a machine learning approach. The full data using all race sectionals was split into two independent datasets: (1) horses that had experienced at least one event (musculoskeletal injury, enforced rest, and/or retirement) and (2) horses that had not experienced any events during the study period. Windows of successive race starts, varying from one to eight, were created. The datasets were then randomly divided (50:50) into train and test sets. The speed and stride characteristics were passed into CatBoost models, along with variables known to affect speed, stride, and injury based on the frequentist methodology described in Part [1], including track condition and surface, race distance, race class, season, number of starters, cumulative race history and rest period parameters. Shapley additive explanation values were used to investigate the extent of contribution of each feature to the final models.

In Part [3] we aimed to assess the effect of bone fatigue accumulation over time on injury risk, using speed (x) for each individual stride taken in every race start. A subset of 405 individual horses that commenced racing and did not undergo enforced rest or retire within the study period were included. Fore-limb vertical force (F; NKg^{-1}) was estimated using the published equation:

$$F = 2.778 + 2.1376x - 0.0535x^2$$

Joint load (σ) was estimated by vertical force, scaled according to the maximum speed and experimentally derived maximal joint surface load (90 MPa) for the metacarpophalangeal joint; the most common catastrophic fracture site. Percentage fatigue life was estimated using a published equation for cycles to failure (N_f) summed across each race start:

$$N_f = 10^{\frac{\sigma - 134.2}{-1\ .1}}$$

Rates of fatigue accumulation were then calculated as the estimated sum of race totals divided by various time periods (days of career, active career, race preparation). Moving averages of bone fatigue accumulation rates over one to five race starts were also calculated. Multivariable Cox proportional-hazard models for each individual horse were generated to investigate associations between total career, active career, preparation and rolling average rates of fatigue accumulation and the instantaneous probability (hazard) of raceday injury as a function of time (days).

The hazard of injury was highest at the second race with 0.039 (95% CI 0.028, 0.054) injuries predicted to occur per race start; decreasing by the tenth race to 0.012 (95% CI 0.009, 0.017) injuries per race start; increasing to 0.019 (95% CI 0.014, 0.025) up to race 35. Expectantly then, through analysis of accumulated bone fatigue, horses were at greatest risk of injury in their first preparation (HR 11.30; 95% CI 3.50, 36.43; P <0.001). The hazard of enforced rest followed a similar bimodal pattern to injury, with risk peaking early in their racing careers; however, the hazard of retirement was more uniform, initially increasing steeply before levelling-out.

As well as the risk of injury changing over a horse's career, we also observed changes in their stride characteristics. Generally, speed and stride length increased over a horses' racing career [0.004 m/s per race start (95% CI 0.001, 0.008; P = 0.008) and 0.002 m per race start (95% CI 0.000, 0.003; P = 0.02), respectively]. But if a decrease in speed was observed, for each 0.1 m/s decrease, the risk of injury increased by 1.18 (95% CI 1.09, 1.28; P <0.001). Similarly, if a decrease in stride length was observed, for each 10 cm decrease the risk of injury increased by 1.11 (95% CI 1.02, 1.21; P = 0.01). Speed and stride length for injured horses declined more rapidly approximately six races prior to injury. Using feature importance in the machine learning approach, enforced rest was less likely to occur with greater increases in stride length two races prior, compared to an individual horse's baseline stride length. Less variation in stride duration across horses' careers predicted retirement. Additionally, medium to high variation in racing speed (throughout horses' careers and during the latest two races) predicted injury, though with overall poor accuracy.

The effect of racing intensity on injury risk across varying timeframes, representing acute, intermediate and chronic race loads was lastly demonstrated in part [3]. Horses with higher rates of fatigue accumulation since their previous start (HR 3.37; 1.01, 11.22; P = 0.048) and over their career had a greater risk of injury (HR 1.80; 95% CI 1.53, 2.11; P <0.001). Fatigue accumulation over four race starts showed a quadratic relationship whereby, compared to a medium rate, low (HR 2.89; 1.39, 6.03; P = 0.005) and in particular, high rates (HR 8.51; 3.35, 21.65; P <0.001) were associated with greater risk of injury and more rapid reduction in survival to the next race.

There were some limitations to these studies. Lack of training data meant that the full skeletal loading history could not be accounted for. The machine learning approach in particular demonstrated poor injury predictability and poor discernability between injury and other adverse outcomes (positive predictive value and sensitivity of enforced rest and injury as a combined outcome: 0.50 and 0.58, respectively; vs injury in isolation: both 0.00). The prevalence of injury over the study period, however, was markedly lower than enforced rest and retirement, resulting in unbalanced event classes which may also have contributed to poor prediction. Further, the prevalence of fatality was too low for epidemiological prediction. These results collectively highlight the value of wearable devices collecting stride data longitudinally. Firstly, to identify changes over time in speed and stride characteristics so as to detect injury early. And additionally, as a tool to estimate skeletal loading in racing over time so that such loads can be modified to reduce injury risk. Rates of workload (and therefore fatigue) accumulation are key contributors to risk of injury, and inclusion of training workload data to more accurately quantify total skeletal loading would be ideal. Therefore, monitoring stride changes and fatigue accumulation in individual racehorses using wearable technology may facilitate early identification of horses at risk of musculoskeletal injury, timely modification of workloads, as well as inform safer training practices.

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SPOTTING INJURY EARLY: TOWARDS IMPROVING THE INTERPRETATION OF SENSOR-BASED MOVEMENT ASSESSMENT IN THOROUGHBRED RACEHORSES

Zoe Chan[1], Bronte Forbes[2], Rebecca Parkes[2,3], Winnie Ho[2,3], Daniel Martel[1], Thilo Pfau[1,4]

University of Calgary, [1]Faculty of Kinesiology, [4]Faculty of Veterinary Medicine, Calgary, Canada
[2]Hong Kong Jockey Club, Hong Kong, China; [3]City University Hong Kong, Hong Kong, China

Retrospective analyses of “in-race” stride parameters and associated injury and training data have shown the potential of reductions in speed and stride length (up to six races prior to injury) for injury prediction (*1*). A prospective prediction of “adverse events” (defined as injuries or enforced rest or retirement from racing) appears less straight forward with a balanced accuracy score of only 44% (*2*). We postulate, that richer “biomechanical models” for quantifying associations between stride parameters and possible confounding factors, such as for example movement direction and surface, are required for improving forwards predictions based on “in-race” data together with injury and training history (*3*).

Lack of movement symmetry is a fundamental characteristic of lameness and expresses as “head nod” or “hip hike” in the trotting horse (*4*, *5*). There is generally little consensus on what level of movement asymmetry needs to be present for a horse to be ‘diagnosed’ as ‘lame’ with animated discussions ensuing about ‘how to define lameness’ (*6*). Interestingly, recent attempts at differentiating between non-lame and clinically mildly lame horses have provided evidence for different asymmetry levels between left and right forelimb lame horses (*7*). This is particularly interesting in racehorses, since depending on the racing jurisdiction, these will train and race consistently in one specific direction, clockwise or anti-clockwise. Here, we hypothesized that horses training and racing in a clockwise direction will show different movement asymmetry patterns from horses training and racing in an anti-clockwise direction even when being trotted in a straight line, but equally when horses are asked to move in tight circles. Second, we hypothesized that skeletal adaptations would ensue in the form of the “inside” and “outside” hooves differing in shape associated with consistent direction-related differential loading patterns. We also hypothesized that, if present, changes in hoof shape would be associated with predictable movement asymmetry patterns. Finally, we expect different patterns as a function of surface, turf versus sand-based, and across straight-line and clockwise and anti-clockwise trot on the lunge.

A comparison of Thoroughbred racehorses training and racing in an anti-clockwise direction at Singapore Turfclub (STC, N=156 horses) with Thoroughbred racehorses training and racing in a clockwise direction at Hong Kong Jockey Club (HKJC, N=151 horses) has provided evidence in support of our first hypothesis (*8*). Chi-squared analysis has shown that there were significantly more (all P<=0.01) left forelimb asymmetrical horses training and racing at HKJC compared to at STC. Mixed model analysis indicated that multiple head (minimum and upwards difference) and pelvic (minimum and upwards difference) movement symmetry variables quantified during in-hand, straight-line trot, showed left asymmetrical movement, i.e. kinematic evidence of reduced force production with the left limbs (*9*, *10*), for the HKJC horses compared to the STC horses. While generally showing small movement symmetry differences during straight-line trot between the two cohorts, the changes were all indicative of a reduced movement (minimum position reached or upward amplitude created) of the “outside limb”, i.e. the limb that is on the outside of the circle during regular training and racing, indicating a reduced force production with the outside limb (*9*, *10*). Previously published kinematic evidence has shown a higher duty factor of the inside limb during gallop – and hence a lower predicted peak force with the inside limb (*11*). Also, trotting horses have been shown to produce a higher outside forelimb peak force (across different surfaces) (*12*). Based on these contradictory findings, we decided to investigate whether there is further evidence of differences between the force produced with the inside and outside limb and whether specific surface-specific adaptations could be measured in racing Thoroughbreds.

In our first study, analysis of hoof shape asymmetries in Thoroughbred racehorses training and racing at HKJC, i.e. in a clockwise direction, were conducted based on solear views taken with a SmartPhone camera and specialist software for hoof width measurements. Distal limb photographs of all four hooves were used to quantify hoof width (at the widest part) at the end of a shoeing cycle in N=186 horses. Paired t-tests compared inside and outside hoof widths for both fore- and hindlimbs. Significant differences (all P<0.01) were found and on average horses exhibited wider inside hooves with mean differences of 2.4 mm and 2.1 mm between the forelimbs and between the hindlimbs.

A further independent-sample t-test for a subgroup comparison between N=65/63 horses with wider inside fore/hind hooves and N=27/28 horses with wider outside fore/hind hooves found significant differences between the two groups for head and withers movement symmetry quantified with inertial sensors. Based on published kinematic and kinetic measurements in lame horses (*9*), these measured movement asymmetries can be assumed to be associated with a specific change in the production of pushoff forces between pairs of contralateral limbs. Overall, this supports our second hypothesis associating skeletal adaptations to movement changes: horses with wider inside forehooves exhibited patterns associated with increased loading of the inside limb (see (*9*, *10*)), and horses with wider outside forehooves showed evidence of increased loading with the outside limb (see (*9*, *10*)).

Finally, our analysis of straight-line, in-hand, trot data with inertial measurement units has indicated increased head movement asymmetry on turf and increased withers movement asymmetry on sand (paired t-tests, all P<0.05). Further subgroup analysis in N=30 horses, utilizing repeated-measure ANOVA with posthoc testing, revealed specific movement adaptations to circular trotting with distinct changes in head movement symmetry between straight-line and anti-clockwise trot, i.e. for the movement direction that this specific cohort of horses, typically training and racing in a clockwise direction at HKJC, is least accustomed to.

Our studies into movement symmetry patterns in Thoroughbred racehorses training and racing consistently in one direction indicate that there are specific movement asymmetry patterns – of on average small amplitude – expected in horses predominantly exposed to uni-directional exercise. This could be important for specifying Thoroughbred-specific movement symmetry values for a) detecting lameness at an earlier timepoint and b) for threshold values for diagnostic anaesthesia and quantifying the efficacy of specific diagnostic blocks (*13*) in the context of racing-specific injuries. The quantified movement adaptations differ as a function of hoof width differences between contra-lateral limbs and are consistent with an increased loading of the wider hoof. Contra-lateral hoof width differences should hence be considered when evaluating gait analysis results and comparing an individual horse's parameters to 'guideline values'.

Finally, the complex movement symmetry changes across surfaces and specifically when moving in a direction opposite to the predominant training and racing direction, indicate that better understanding of gait, surface and movement direction-specific characteristics could improve the accuracy of predictive models (*1, 2*) by incorporating richer biomechanical knowledge into these models. Based on our findings to date, we propose to emphasize the creation of surface and movement direction related models with quantitative inputs for surface characteristics and track geometry.

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LOCOMOTION CHANGES INDUCED BY SURFACE FIRMNESS OF A CROSS-COUNTRY COURSE: PILOT STUDY ON 3 HORSES

Nathalie Crevier-Denoix[1], Lauriane Fayaubot[1], Lotte Kraus[2], Florian Delaplace[1], Jean-Marie Denoix[1], Philippe Pourcelot[1]

[1] INRAE, Ecole Nationale Vétérinaire d'Alfort, 957 BPLC, Maisons-Alfort, France ; [2] CLJ Equine Healthcare, Ooldselaan 9a, Laren, The Netherlands

Characterization of equestrian surfaces has made significant progress over the last ten years thanks to dedicated testing devices. However, the effects of surface characteristics on the horse's locomotion during high intensity exercise yet lack assessment through experimental studies.
IMU-based wearable sensors are increasingly used in equine sports medicine, allowing measurement under real sport conditions. However most of the currently available devices have not been validated for measurement accuracy and precision, although this is critical when using these tools for scientific purposes. To the authors' knowledge, the *Alogo Move Pro* sensor system is the only device that has been compared with an optical motion capture system for jump performance assessment (Guyard et al., 2023). It has then been applied for detecting kinematic signs of fatigue in eventing horses (Burger et al., 2024).
In the present pilot study, the *Alogo Move Pro* has been used for characterizing stride parameters of 3 eventing horses cantering on a soft-to-deep versus a firm turf areas of a cross-country course. It was hypothesized that surface firmness would have an impact on the main canter stride variables.

Three eventing horses (Selle Français geldings, 7 to 9 years old), ridden by their respective riders and competing in the same national level 3-day event (Elite Pro 4, 3000 m, 24 efforts) were included.
The day prior to the cross-country session, surface testing was performed throughout the course. Both a 2.25 kg Clegg impact soil tester (CIST) and the patented device *Equine Track Tester* (ET2; Crevier-Denoix et al., 2021; Munoz-Nates et al., 2022) were used. A CIST test consists in 3 successive impacts at a given location; resulting maximal deceleration is expressed in G. The ET2 (Figure1) has been designed to simulate the action of an equine forelimb on the ground under sport conditions, both in terms of maximal vertical force and loading rate. A test consists in the dropping of a 110 kg mass, sliding along a vertical axis connected with a hoof-shaped impactor in contact with the ground. After a first phase of free fall, the mass pushes the axis-impactor system down inducing ground loading, the rate of which is decreased by the tension of an elastic device which mimics the action of the equine musculotendinous system. From each ET2 test, the following variables were determined: maximal vertical force, maximal sinking (vertical displacement of the impactor), vertical loading rate (slope of the force-time curve).
Within the first third of the course, two flat areas of natural turf of about 150 m long each, situated at two different altitudes, could be discriminated according to their firmness, especially as the weather had been particularly rainy the days before the competition: a wet, "soft"(deep), versus a "firm" area.
On each area, CIST and ET2 measurements were performed on 3 locations (about 75 m apart).
On the competition day, the *Alogo Move Pro* sensor (Alogo Analysis SA, Renens, Switzerland) was placed in a custom-made pocket attached to the saddle girth over the sternum (Figure1). Accelerations (on the x, y, z axes) were recorded at 100 Hz. Position and speed were derived from these measures, then the following variables were computed for each stride: speed, maximal vertical acceleration, maximal vertical amplitude, stride length and duration, as well as mean longitudinal (pitch) and lateral (roll) angles.
Given the small number of CIST and ET2 measurement sites on each area, and as the difference between areas was very clear (see below), only the Alogo measurements, on which this study is focused, were statistically tested. The two areas were compared using linear mixed-effects models, considering the different horses and allowing adjustment of speed. All analyses were performed using SAS software ($p<0.05$).

Mean (SD) of the CIST impacts on the firm area was 103.2 (22.2) G vs. 45.5 (20) G on the soft one. According to the ET2, the maximal vertical force was about 28% larger on the firm area: 10 429 (289) N vs. 8 129 (1 222) N on the soft area, the maximal sinking of the hoof-shaped impactor was 79% smaller on

firm: 9.9 (5.1) mm vs. 47.3 (20.4) mm on soft, and the overall vertical loading rate was 55% greater on firm: 236 032(16 219) N/s on firm vs. 152 741 (41 118) N/s.
Among the Alogo variables, the horse's speed was significantly higher on the firm area (+10.5%, 521 vs. 471 m/min; p<0.0001). After speed adjustment, differences in stride length was no longer significant between areas (+1.5% on firm vs. soft, p=0.10), but stride duration tended to be larger on firm (+1.7%, p=0.08) (Figure 2). Conversely, maximal vertical acceleration and vertical amplitude of the stride were still larger on the firm area (+24% and +17%, respectively; p<0.0001). The mean longitudinal balance showed a significantly larger forward (descending) incline on the firm surface (+29%, p=0.002), and the absolute value of the lateral balance was lesser (-40%, p=0.0003), meaning less roll of the trunk, on the firm surface.

This pilot study demonstrated that the *Alogo Move Pro* device was able to reveal subtle kinematic changes during the stride, even after adjustment on speed, such as maximal vertical acceleration, vertical amplitude of the trunk movement, as well as average pitch angle (longitudinal balance leaning forward), all three being increased on the firm area compared with the soft one. These effects of the ground surface on the locomotion variables suggest a more powerful and efficient propulsion on the firm surface. They should be taken into account when interpreting locomotion pattern of horses cantering on surfaces of variable firmness, such as those encountered on cross-country courses. Besides, these data could help in evaluating the adequacy of a cross-country course to prevent the risk of injury in competing horses.

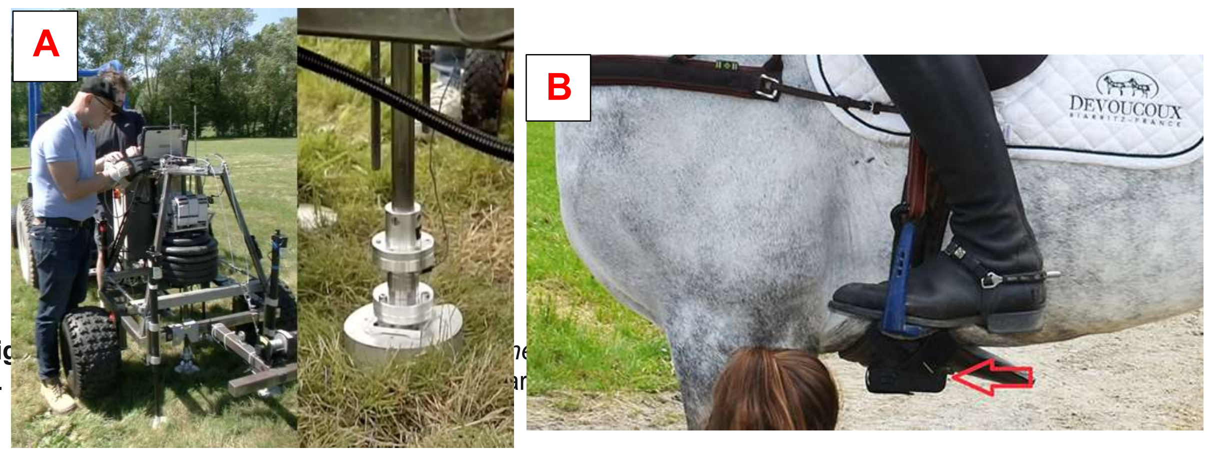

Fig
B.

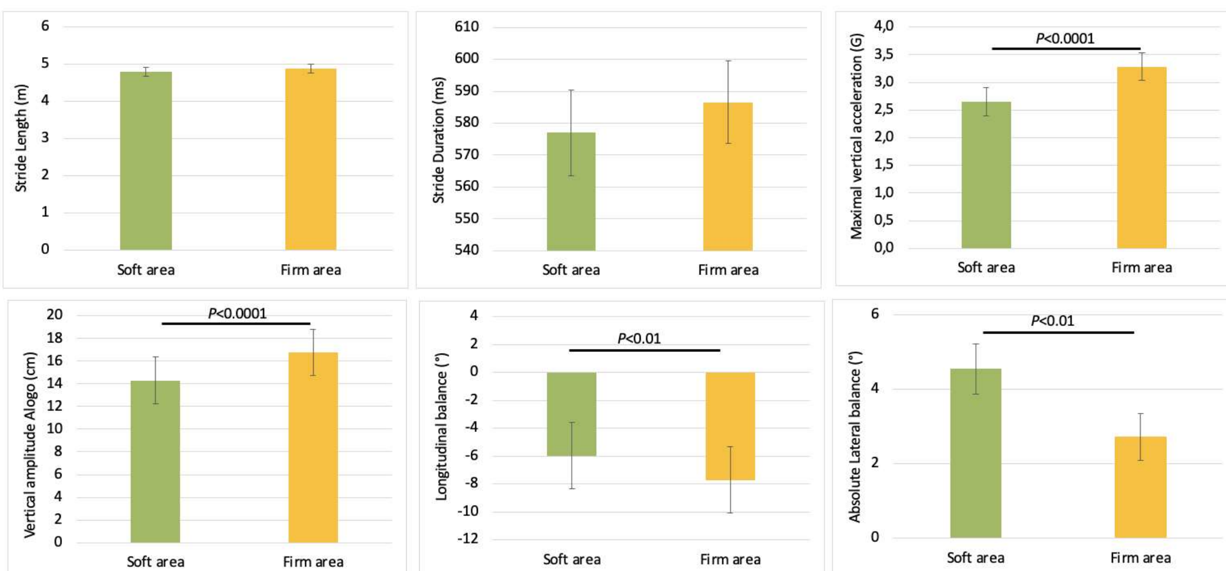

Figure 2: Stride variables on the soft (green) and firm (orange) areas.
Each diagram presents the least square means and 95% confidence intervals.

SESSION III. Cardiology/Respiratory

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CARDIAC ARRHYTHMIAS DURING INTENSE EXERCISE IN THOROUGHBRED RACEHORSES: INCIDENCE AND ASSOCIATION WITH RACE PERFORMANCE

E. van Erck-Westergren[1], S. O'Connor[2], B. Stewart[2], G. Dubois[3], J. Morton[4], K. Hinchcliff[5], F. ter Woort[1]

[1] Equine Sports Medicine Practice, Waterloo, Belgium; [2] Hong Kong Jockey Club, Department of Veterinary Clinical Services, Sha Tin Equine Hospital, NT, Hong Kong; [3] Arioneo, Paris, Île de France, France; [4] Jemora Pty Ltd, East Geelong, Victoria, Australia; [5] Trinity College, Melbourne, Victoria, Australia.

Cardiac arrhythmias are common during maximal exertion in equine athletes, with their severity ranging from clinically benign to potentially fatal. While atrial fibrillation (AF) and complex ventricular arrhythmias are widely acknowledged as serious, isolated ectopies are generally considered of little consequence. However, their actual incidences and associations with subsequent race performance, especially for ECGs recorded under field conditions, remain poorly understood. With advances in wearable technology, extensive ECG data can now be collected during high-intensity track exercise, offering new opportunities for detailed analysis.

The objectives of this retrospective case-control study were to (1) describe the incidences of various arrhythmias in Thoroughbred racehorses during high-intensity field sessions and (2) to explore associations between ECG variables and subsequent race performance.

Data were collected from May 2021 to July 2022 in Thoroughbreds trained by 1 trainer, using Equimetre technology (Arioneo, France) to record speed and ECG data during training gallops and barrier trials ("exercise events"). Case and control race starts were selected from races by horses with a recent preceding ECG at an exercise event. Control starts were selected from those where the horse was placed in the top 3 (good performance) and case starts were selected from those where the horse finished in the last 3 (poor performance). ECGs and associated training data recorded during the days or weeks preceding the race were used for analyses. Only exercise events where the maximum speed was > 55 kph and the ECG recording was of good or excellent diagnostic quality were used for analyses. The following ECG characteristics were recorded: timing of arrhythmia (during each of warm-up, maximal, fast and slow recovery exercise phases), number of ectopies during each phase and type of ectopies (narrow, wide, association with a compensatory or non-compensatory pause, complex, paroxysmal atrial fibrillation).

In total, 151 case and 278 control starts and the associated prior ECGs from 72 horses were used in analyses. Most horses had between 2 and 9 race starts and associated ECG recordings included. Arrhythmias were common, with only 23% of ECGs free of arrhythmias. Arrhythmic events were very common during the maximal phase of exercise (@max; 63% of ECGs), including complex patterns such as triplets, quadruplets (7.0%), and complex or paroxysmal AF (2.3%). Ectopy timing differed between exercise types, having generally earlier onset in shorter barrier trials and training gallops (<140 s) and mid-event onset in longer training gallops (>140 s). No substantial correlations were evident between numbers of arrhythmic events occurring @max and those recorded at any other exercise phase. Intraclass correlation coefficients adjusted for maximum heart rate and maximum speed during exercise event showed that while the number of arrhythmic events varied between ECGs within horse, it was also moderately clustered by horse (intraclass correlation coefficients during warm-up (0.63; 95% confidence interval (0.54-0.72) and @max (0.42; 0.30-0.55).

Univariable analyses revealed that the odds of a bottom 3 finish increased with higher ectopy counts during the maximal phase of the prior exercise event (OR 1.18 for each additional ectopy; OR 2.26 per 5 additional ectopies, p = 0.002) and were also associated with numbers of complex ectopies such as couplets (OR 1.98 for each additional couplet; OR 30.56 per 5 additional couplets, p = 0.011).

In conclusion, this exploratory study identified associations between numbers of arrhythmias during maximal exertion at training gallops and barrier trials, and poor subsequent race performance. Monitoring arrhythmias using wearable ECG technology could serve as a valuable tool for detecting problematic arrhythmias during training, predicting race outcomes, and ultimately enhancing equine health and welfare in the high-stakes racing industry.

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EVALUATION OF THE ‘EQUIFIB’ APP WITH POLAR H10 FOR AUTOMATIC ATRIAL FIBRILLATION DETECTION IN HORSES

Gunther van Loon, Glenn Van Steenkiste

Equine Cardioteam Ghent, Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

This study investigates the use of a mobile application, EquiFib, developed on the Flutter platform and made freely available by the Equine Cardioteam Ghent, for detecting atrial fibrillation (AF) in horses using the Polar H10 heart rate monitor. The app employs an integrated neural network (NN) to conduct on-device analysis of 30-second electrocardiogram (ECG) segments. The conclusion is immediately displayed on screen and can be sent by email for verification. The ECG is uploaded to a server for scientific purposes and further training of the app in future.

Over six months, data from 1151 recordings were collected across 38 countries by 164 users, with a median of 2 recordings per user. An individual observer categorized 823 (71.5%) of these as sinus rhythm (SR), 104 (9.0%) as AF, and 224 (19.4%) as not classifiable. The NN underwent ten-fold cross-validation on all recordings post-collection, aimed at enhancing the original algorithm developed using Televet recordings, which previously yielded suboptimal results. The newly validated NN demonstrated an overall accuracy of 96%. Table 1 illustrates the detailed performance metrics, including the positive predictive value for SR and AF detection, alongside sensitivity and specificity measures. These results underscore the app’s enhanced capability in accurately discerning AF and detecting low quality ECGs, providing valuable insights into the presence of AF. A limitation of the study was that there was no control on who made the recording (veterinarian, owner), if the recording was performed correctly (at rest and not during exercise, moistened skin, electrodes correctly positioned) or whether the recording was actually from a horse.

It was concluded that the EquiFib app can contribute to animal welfare and rider safety as it is a promising tool for early detection of AF occurrence or recurrence in equine patients. The cheap, easily available H10 Polar heart rate transmitter in combination with a free app stimulates owners to apply it. As the user-friendly technology allows it to be used on a daily basis, stall side, by the owner, the app might address potential issues in equine sport linked to AF. The app is currently integrated in the veterinary cardiac workup (diagnosis, monitoring) in our clinic and empowers horse owners to perform convenient home-monitoring of their horse’s cardiac rhythm.

Table 1: Ten-fold cross-validation detailed performance metrics of the neural network from the EquiFib app by the Equine Cardioteam Ghent.

	Positive predictive value (%)	Sensitivity (%)	Specificity (%)
Sinus rhythm	95.6	95.9	88.7
Atrial fibrillation	94.7	76.7	99.5
Artefacts	85.6	91.1	96.1

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FEASIBILITY OF ACQUIRING ECGS REMOTELY AND PREVALENCE OF ARRHYTHMIAS IN THOROUGHBRED RACEHORSES DURING HIGH-INTENSITY WORK

Mary Durando[1], Sian Durward-Akhurst[2], Hamid Tavanaeimanesh[2], Cristobal Navas de Solis[3]

1. Equine Sports Medicine Consultants, Newark, Delaware, USA; 2. Department of Veterinary Clinical Sciences, University of Minnesota, Minnesota, USA; 3. University of Pennsylvania School of Veterinary Medicine, New Bolton Center. Pennsylvania, USA

The specific aims of this study were to 1- determine if high-quality ECG of horses during high-intensity work can be evaluated remotely after recordings are obtained from fitness tracker devices placed by personnel with no medical training in a racetrack setting as part of their daily routine; and 2- describe the frequency and types of arrhythmias in these horses. This study is part of the evaluation of a screening program using wearable technology with the long-term objective to decrease exercise-associated sudden death in Thoroughbred racehorses. Institutional Animal Care and Use Committee approvals were obtained.

A convenience sample of trainers in the United States were invited to participate in this study, and horses were selected by each trainer via convenience sampling. During the first 4 months of the study 218 horses were enrolled with the goal of obtaining 1 high-intensity exercise recording every 7 days using the Equimetre/Arioneo “all-in-one” fitness tracker device. This device has GPS and motion sensors that can record and calculate speed, distance traveled, and stride frequency and length, along with electrodes that can record heart rate and cardiac rhythm. The dorsal electrode was placed caudal to the dorsal aspect of the scapula, ventral to the withers on the left side, and the ventral electrode was placed near the sternum on the left side. This resulted in a modified base-apex lead. All data were saved to cloud storage for later analysis. ECGs were later analyzed using Kubios Premium version 4.1.2 (Finland).

Two readers reviewed recordings. ECG recordings were divided into high-speed exercise (duration from the onset of rapid acceleration of heart rate to the onset of rapid heart rate decline) and early recovery (first 2 minutes of rapid heart rate decline). High-speed exercise was >30 mph (<15 second/furlong) breeze. Recordings with 90% or more of the peak exercise portion containing clearly identifiable QRS complexes were considered readable and included for analysis. All ECGs were manually corrected for mislabeled complexes after automated analysis with a % prematurity set at 5%. Electronic calipers were used to determine the RR of normal heart rate in the beat preceding the premature complex, the coupling interval (RR of premature complexes), and if a compensatory pause was present. Premature complexes were defined as an RR coupling interval of >/=5% prematurity compared to the preceding RR. Couplets were defined as 2 consecutive premature complexes, triplets were defined as 3 consecutive premature complexes and tachycardia as >3 consecutive premature complexes. Complex arrhythmias were considered to be triplets, tachycardia, atrial fibrillation, or R-on-T configuration. Because the Equimetre only records one lead, no attempt was made to classify complexes as supraventricular or ventricular. However, the QRS morphology was described as narrow or wide complex or reverse polarity by subjective comparison of the premature complexes to the surrounding sinus-generated complexes. Statistics were descriptive and summary statistics displayed according to the distribution of the data.

Six hundred and sixty training sessions from 218 horses were available in the online database after 4 months. These recordings were obtained using 34 fitness trackers by 17 trainers (1-4 tracker/trainer). At the time of enrolment, two hundred and fifteen horses had no reported clinical concerns, and 3 had clinical concerns that were ‘fading in the last furlong,’ paroxysmal atrial fibrillation, and ‘collapse after training.’ Two hundred and ninety ECG recordings were evaluated and 227 met readability criteria (78.3%). In 27 of these recordings, 30mph was not achieved, and 200 readable ECGs from 87 horses (range 1-14 ECGs/horse) were used for descriptive statistics. The peak heart rate was mean±SD 222±9/min when measured electrocardiographically as the average of the instantaneous heart rate of the 5 shortest RR excluding premature complexes and 218±8/min when measured automatically by the fitness tracker (average of 15 seconds and automatic elimination of outliers). The duration of the high-intensity exercise portion was 166±56 seconds.

There were arrhythmias in 169 recordings (84.5%) from 77 horses (88.5%). There were arrhythmias during high-intensity exercise in 154 (77%) recordings from 73 horses (83.9%) and during recovery in 96 recordings (48%) from 48 horses (55%). There were complex arrhythmias in 44 recordings (22%) from 25 horses (28.7%). The number of premature complexes during exercise was median (IQR), [range] 3, (1-8) [0-37], and the total number of premature complexes including exercise and recovery was 4 (1-10.5), [0-39]. Forty-six recordings (23%) from 20 horses (23%) had 10 or more premature complexes during exercise, and one horse developed paroxysmal atrial fibrillation. The three horses with clinical concerns at the time of enrolment did not show the reported signs after enrolment but 2/3 displayed complex arrhythmias. No horse developed collapse or sudden death.

It was feasible to obtain ECGs in horses with a workflow that did not need a veterinarian in the barn. The goal of obtaining one recording per horse per week was not achieved. The % of readable ECGs and the number of recordings per horse are potentially acceptable for a screening program, but this conclusion is speculative as the frequency of electrocardiographic premonitory signs for collapse and death is unknown in the horse. A large-scale monitoring program would need the development of automatic ECG analysis tools. The % of recordings and horses with complex arrhythmias was higher than expected when compared to previously reported normal populations of racehorses and to populations presented with signs of poor performance or cardiovascular disease. The goal to obtain ECGs during high-intensity work, the inclusion criteria, and the selection for analysis of electrocardiograms based on speed achieved during training are potential factors in this difference. The evaluation of associations of arrhythmias with performance, collapse, and sudden death needs a large population and this is the next step in this ongoing project.

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EFFECTS OF INDUCED LARYNGEAL HEMIPLEGIA ON AEROBIC CAPACITY AND EXERCISE-INDUCED PULMONARY HEMORRAGE IN THOROUGHBRED RACEHORSES

S. Massie[1], R. Sides[2], W. Bayly[2], R. Léguillette[1]

[1]Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada; [2]College of Veterinary Medicine, Washington State University, Grimes Way, Pullman, Washington, USA

Exercise induced pulmonary hemorrhage (EIPH) is postulated to worsen as inspiratory pressures increase, as seen in recurrent laryngeal neuropathy (RLN). Aim: to induce RLN in healthy Thoroughbred racehorses and assess aerobic capacity ($\dot{V}O_2$), breath-by-breath analysis (*Fig1*) and EIPH. It was hypothesized that RLN would negatively impact $\dot{V}O_2$peak and ventilatory parameters, resulting in more severe EIPH.

Eight healthy horses performed a randomized crossover study (Animal Care #6815; consent provided by owners), consisting of two maximal exercise tests: control and RLN (n=3 treadmill; n=5 track). RLN was induced by injecting Bupivacaine (4.5 ml) into the cricoarytenoideus dorsalis muscle under ultrasound guidance. Overground endoscopy, heart rate, $\dot{V}O_2$peak, and ventilatory parameters (respiratory rate, tidal volume, peak inspiratory and expiratory flow, and minute ventilation), post-exercise EIPH (tracheal endoscopy and bronchoalveolar lavage) and blood lactate were measured. Control and RLN were compared (Wilcoxon signed-rank paired T-test; $P<0.05$).

RLN was induced in 8/10 runs (Grade-B=1; Grade-C=7) but not sustained in one run. Laryngeal function was restored within 1 hour. Control and RLN $\dot{V}O_2$peak (153 ml/kg.min, 143 ml/kg.min respectively) and ventilatory parameters were significantly different. Heart rate and lactate were similar between runs (control:189 [178, 201] bpm; 10.0 [1.7, 13.0] mmol/L; RLN: 190 [171, 200] bpm; 6.1 [1.9, 17.0] mmol/L). Endoscopic signs of EIPH were present in four horses following control runs (Grade-1=3; Grade-2=1) and six horses following RLN (Grade-1=3 and Grade-2=3). No differences were observed between BALrbc ($P=0.68$).

This study confirms the feasibility of an induced RLN experimental model and its deleterious effects on $\dot{V}O_2$pk and spirometry.

Figure 1: *a) RLN; b) overground endoscope and ergospirometry facemask; c) power units*

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VALIDATION OF A SMART TEXTILE DEVICE FOR RECORDING ELECTROCARDIOGRAMS IN STANDARDBRED DURING HIGH-SPEED EXERCISE

Kapusniak, A.[1], Nath, L.[1], Hitchens, P.[2], Bailey, S[2]., McCrae, P.[3] and Franklin S.[1]

[1]School of Animal & Veterinary Sciences, University of Adelaide, South Australia, Australia; [2]Melbourne Veterinary School, University of Melbourne, Victoria, Australia; [3]Myant Inc., Toronto, Ontario, Canada.

The purpose of this study was to evaluate the use of a novel smart textile band (Skiin Equine; Myant Inc.) for recording of electrocardiograms (ECGs) during strenuous exercise.

ECGs were recorded simultaneously using the Myant Skiin Equine device and a referent device (Televet II) in 25 healthy Standardbred racehorses in training, during high-speed exercise. GPS data was extracted to determine maximum speed for each trial. ECGs were evaluated using Kubios software, where the percentage of artefact was recorded, and the diagnostic quality of the traces assessed by a blinded observer. Maximum heart rate (HR_{max}) was calculated over the last 30 seconds of maximal exercise and compared between devices. A chi-squared test was used to determine whether there was a significant difference in the proportion of recordings with >10% artefact between the two devices. Intraclass correlation coefficient (ICC) & Bland-Altman analysis (bias, 95% limits) were used to compare HRmax between devices.

ECGs were reviewed for arrhythmia detection. Premature depolarisations (PD) were identified based on R-R deviations of >20% at rest, >5% during exercise and >10% during recovery. They were further classified by QRS morphology as having either narrow or wide appearance.

The timing of ECG files was separated into 5 phases: rest, submaximal exercise (HR <190bpm), maximal exercise (HR>190bpm), recovery 1 (the first 2min post-exercise) and recovery 2 (from recovery 1 until the HR returned to 80bpm). ECGs with >10% artefact overall, or that were considered non-diagnostic during maximal exercise, were excluded from further analysis. Rhythm disturbances were classified as either isolated PDs (one or more premature depolarisations that occurred singly, narrow or wide) or complex (two or more PDs that occurred in pairs, triplets or salvos, on at least one occasion). Agreement on classification was compared between devices using a Cohen's Kappa coefficient and the total number of PDs were compared using a paired t-test.

The median (IQR) peak speed during exercise was 13.9m/s (IQR=0.75m/s). One Skiin ECG and 2 Televet ECGs had artefact >10%. Mean (SD) HRmax was 224.7±7.6 for Skiin and 223.7±7.4bpm for Televet. The Skiin Equine smart textile showed strong agreement with the Televet for HR max measurements (ICC=0.963, mean bias -1bpm).

A total of 21 pairs of ECGs were used for the arrhythmia detection analysis. Three pairs were excluded due to artefact >10% and one was excluded due to artefact in maximal exercise. Rhythm disturbances were identified in 18 horses (18/21; 86%) (7 isolated; 12 complex). The agreement between devices for arrhythmia classification (no arrhythmia, isolated PD, complex PD) was 81% (k=0.693, SE 0.157). There was no significant difference between devices for the total numbers of PDs identified during the exercise tests (Skiin = 5.14 ± 4.45 and Televet = 4.43 ± 5.04; t(20)=0.82, p=0.42).

In conclusion, the Skiin Equine device provides a reliable ECG trace for recording of ECGs during strenuous exercise, potentially allowing its application to widespread monitoring of horses during training and racing.

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FEASIBILITY AND APPLICATON OF A SMART PHONE BASED ELECTROCARDIOGRAM FOR SCREENING IN THE THOROUGHBRED RACEHORSE.

Morgan Jessica M.[a], Hopkins Savannah[a] Batten Casille[b], Maas Lauren T[a], Navas de Solis Cristobal[c], Lehman Mallory[a], Dupuis-Dowd Florence[a], Behar Jessica[a], Buecken Welf[a], Gentille Samantha[a], Goyette Francesca[a], Scott Farris[a], Webb Ryleigh[a]

[a] School of Veterinary Medicine, University of California, Davis Department of Medicine and Epidemiology Davis, CA; [b] Golden Gate Fields, Berkeley, CA; [c]New Bolton Center, University of Pennsylvania, Department of Clinical Sciences, Kennett Square, PA

Exercise associated deaths in racehorses have profound effects on the safety and public perception of equestrian sport. Sudden death, defined as acute collapse and death in an apparently healthy horse during or immediately after exercise in the absence of catastrophic orthopedic injury, represents 9-19% of racing associated deaths. The most common explanations for sudden death in the horse are attributed to cardiorespiratory disease, including idiopathic blood vessel rupture, pulmonary hemorrhage, and dysrhythmias. In human athletes, some form of pre-participation cardiovascular screening has been effective at reducing deaths during exercise and is recommended by several medical and sporting organizations. This study set out to evaluate the feasibility of smart-phone based electrocardiogram (ECG) screening for use in the Thoroughbred racehorse prior to and immediately post-exercise. The secondary goal of this study included defining reference intervals for smart-phone based ECG heart rate, PQ interval, QRS duration and QT interval in the pre- and post-exercise period.

One hundred and one Thoroughbred racehorses in active race training in Northern California were enrolled in the study between May-November 2023. Horses were housed in barns on a racetrack in California and were enrolled in the study through a collaboration with a regulatory racetrack veterinarian and available trainers. All horses underwent a physical exam and cardiorespiratory screening at rest and again following a timed work. Resting exams were performed the afternoon prior to a timed work to maximize the opportunity for horses to be fully recovered from morning exercise. Post-exercise exams were performed as promptly as possible once the horse had returned to the barn and recovered sufficiently to stand for the examination. ECGs were performed in the horse’s stall, barn aisle or grooming area. Electrocardiography was performed by a single veterinarian and consisted of 30 second recordings with a KardiaMobile two lead device, which was placed on the left thorax. ECG recordings were saved with a smart-phone onto the KardiaMobile application. ECG measurements were performed manually using Image J. Measurements were performed on three consecutive cardiac cycles within the 30 second recording and mean values were reported. In cases where an ECG was repeated during a single observation period due to poor quality or loss of signal before 30 seconds, only the highest quality ECG obtained was used for measurements. Owner and trainer consent were obtained for the use of all animals in the project and the International Animal Care and Use Committee (IACUC) was consulted regarding the appropriate approvals for these non-university-owned animals.

Statistical analyses were performed on commercially available GraphPad Prism Software (GraphPad Prism 9.3.1, GraphPad Software, San Diego, CA). A Shapiro-Wilks test was used to determine the normality of the data. A paired t-test was used for the comparison of normal values in the pre- and post- work periods and a Wilcoxon matched-pairs signed-rank test was used for the comparison of non-normal values in the pre- and post-work periods. An ANOVA was used to compare heart rate by furlongs worked in the post-exercise period. $P < 0.05$ was considered significant.

A total of 101 Thoroughbred racehorses were enrolled in this study and received an ECG during the rest period. Ninety-six of the horses performed timed works and received an ECG post exercise. Of the five horses who did not perform a timed work, one was due to the identification of ventricular premature complexes during the resting exam. The remaining horses were not worked at the trainer’s discretion and the decision was unrelated to the study findings. Obtaining a 30 second ECG required a median of 52 seconds (Interquartile range (IQR) 36-69) in the resting examination and 46 seconds (IQR 37-67) in the post exercise examination. This included some instances where multiple ECGs were obtained due to loss of signal or excessive noise ending the recording prematurely. After exclusion of ECGs of inadequate quality (1) or inadequate labeling (6), ECGs from 89 horses were used for further analysis. The final sample included 30 mares/fillies and 59 males (58 geldings, 1 colt) aged 2-8 years old (median 4, IQR 3-5). They performed timed works 27.2-64.0 seconds (median 50.2, IQR 48.6-61.6) over 2-5 furlongs (median 4, IQR 4-5). All horses except the horse with ventricular ectopy noted during the resting examination were determined to be in normal sinus rhythm. One horse had atrioventricular block noted at rest. Heart rate during the resting examination ranged from 25-85 beats per minute (BPM) with a median of 40 BPM. Heart rate during the post exercise examination ranged from 42-132 beats per minute (BPM) with a median of 78 BPM. Heart rate was significantly increased post exercise (P<0.0001) and increased in horses working 5 furlongs compared to those working 4 furlongs (P=0.02). Resting and post-exercise ECG measurements are reported in Table 1 for future reference.

Smart-phone based ECG screening was practical and of diagnostic quality when performed at the race track in both resting and post exercise conditions in the Thoroughbred. The recordings were acquired in under a minute making this a practical approach for safety screening. A single horse was flagged for the presence of an arrhythmia in this convenience sample of horses in race training, suggesting this screening protocol may have utility in identifying previously undiagnosed arrhythmias. Establishing expected heart rates for Thoroughbreds in training was a secondary goal of this study as these may aid in identifying horses with inappropriate heart rates during screening. Based on the differences in post-exercise heart rate between horses that worked 4 and 5 furlongs it is likely that unique guidelines for expected heart rate will be needed for specific racing conditions. Based on the experience and data gained in this study the authors believe implementation of an ECG based screening during pre-race examinations is feasible and has potential to identify at risk horses. Post-examinations may be helpful particularly in horses that under-perform and may have developed arrhythmias during the race although not observed in this study. However, expected heart rates will vary based on the type of work performed.

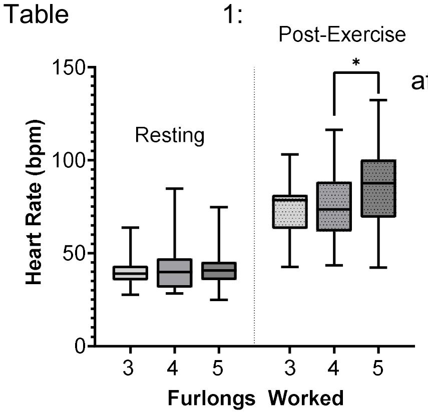

Figure 1: Heart rate measured on a smart-phone based ECG recording at rest and post-exercise for 89 Thoroughbred racehorses worked 3, 4, or 5 furlongs in an official timed work. * =P<0.05

Table 1: Electrocardiographic measurements for 89 Thoroughbred racehorses reported as median and interquartile range (IQR) acquired the afternoon before (resting) and after a timed work (post-exercise).

	Resting Median (IQR)	**Post-Exercise** Median (IQR)
PQ (ms)	250 (220 – 300)	200 (180 – 230)
QRS (ms)	100 (100 – 110)	100 (90 – 110)
QT (ms)	460 (430 – 480)	390 (350 – 440)
HR (bpm)	40 (35 – 46)	78 (65 – 93)

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USE OF ATRIO SOFTWARE TO ANALYSE ECGS FROM HORSES WITH AND WITHOUT A HISTORY OF ATRIAL FIBRILLATION

Nath, L.[1], Kapusniak, A.[1], Mohamed, R.[2], Franklin, S.[1]
[1] School of Animal and Veterinary Sciences, University of Adelaide, South Australia, Australia; [2]Alerte Digital Health, Melbourne, Victoria, Australia

Atrial fibrillation (AF) is the most common performance limiting arrhythmia in Thoroughbred racehorses. The vast majority of cases are paroxysmal, and AF episodes are challenging to capture in the absence of wearable or rapidly applied electrocardiographic (ECG) devices. As a result, AF can often go undiagnosed as a cause of poor performance. Algorithms featuring complexity analysis have been applied to sinus rhythm ECGs and were shown to discriminate between horses with and without a history of AF. However, the features of resting and exercising ECGs from horses with and without a history of AF are relatively unexplored.

The purpose of this study was to use novel software to record features of resting and exercising ECGs from race fit horses in sinus rhythm. Cases were horses that had previously had a diagnosis of AF and had returned to racing following conversion (spontaneous or medical cardioversion) to sinus rhythm. Controls were horses with no history of AF.

Exercising ECGS were collected from Thoroughbred racehorses during fast work under saddle as part of routine training procedures using either the Televet or Equimetre devices and speeds were obtained from device recorded GPS data. Holter ECGs were collected over approximately 12 hours with the Televet. ECGs were imported into the Atrio software for viewing and recording of ECG features. Atrio software is capable of displaying 3 leads simultaneously (figure 1) and provides autodetection and labelling of normal and abnormal QRS complexes in addition to normal and abnormal rhythms (figure 2). Manual correction of incorrectly labelled complexes is facilitated by the software. ECG features detected include: premature complexes, atrioventricular block and aberrant conduction. Heart rate variability analysis (HRV), was performed with the software and comprised of SDNN and RMSSD. Normality of data was determined using the Shapiro-Wilk test. Results were compared between cases and controls using an unpaired t-test for normally distributed data and a Mann-Whitney test for non-normally distributed data.

There were no differences between cases (N=5, aged 4.8 ±1.2 years) and controls (N=4, aged 4.5 ± 0.8 years) in peak speed (64 ± 3.0 kph cases vs 63 ± 3.0 kph controls, P=0.45) or gallop distance (1847 ± 338m vs 1916 ± 268m controls, P=0.68). In the exercise test, there were no differences in maximal heart rate (250 ± 65 vs 219 ± 4.7 controls, P=0.47), narrow premature complexes (median [range]) {(2 [0-3] controls vs (0.5 [0-2] controls, P=0.38}, wide complexes (0 [0-32] cases vs 0 [0-7.5] controls, P =0.84). In the holter ECG, there were no differences in minimum HR (29 ± 7 BPM cases vs 27 ± 7 BPM controls, P= 0.71), second degree atrioventricular block (0[0-8] cases vs 0[0-39] controls, P= 0.71), supraventricular ectopy (2.5 [0-11]) cases vs 2 [1-962] controls, P=0.71), ventricular ectopy/aberrancy (0.5 [0-961] cases vs 0[0-0] controls, P=0.410. There were no differences in HRV between cases and controls either during exercise for SDNN (370 ± 218 cases vs 374 ± 131 controls, P=0.73) and RMSSD (254 ± 372 cases vs 41 ±19 controls, P= 0.41) or during holter monitoring for SDNN (245 ± 40 cases vs 259 ±100 controls, P=0.86) and RMSSD (95 ± 38 cases vs 149 ± 91 controls, P=0.63). One horse with a history of AF developed AF in the exercise test and another horse with a history of AF had suspected ventricular pre-excitation.

Low numbers of horses in each category limited the statistical power to detect differences between groups.

In conclusion, Atrio software was useful for reporting ECG features from horses during both exercise and holter monitoring. There is wide individual variation in the ECG features from horses with a history of AF and normal horses. Underlying conduction disturbances such as ventricular pre-excitation might predispose horses to the development of AF during exercise. Additionally, strenuous exercise might precipitate AF in vulnerable horses.

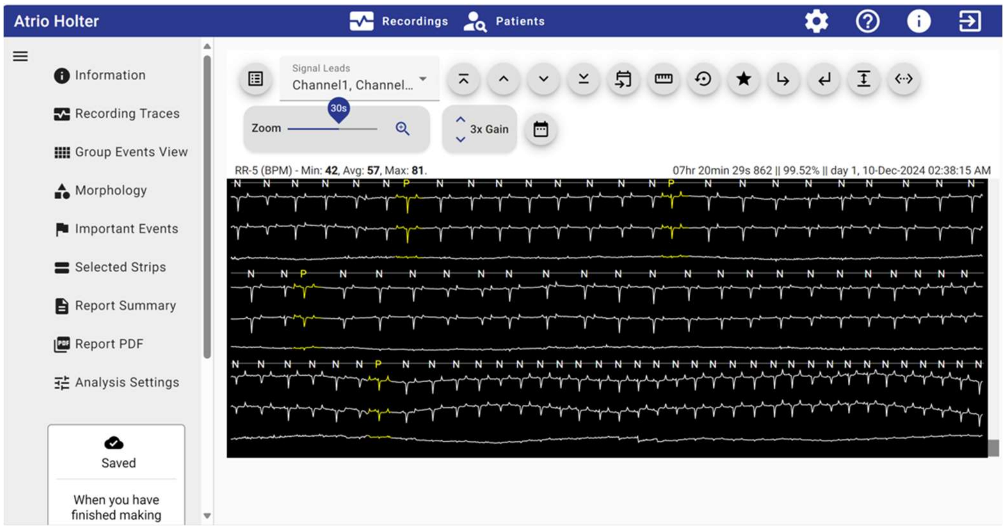

Figure 1. Display of 3 lead Televet ECG holter recording with labelling of supraventricular premature complex.

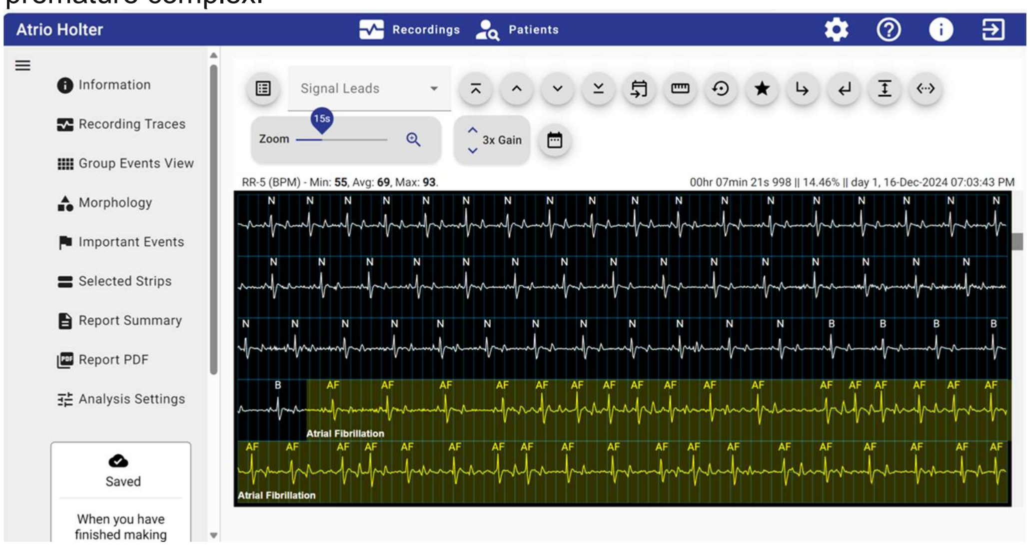

Figure 2. Display of single lead Equimetre ECG exercising recording with labelling of atrial fibrillation rhythm.

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SHORT BURSTS OF PAROXYSMAL ATRIAL FIBRILLATION DURING EXERCISE IN RACE HORSES: CASE DESCRIPTION, ECG CHARACTERISTICS AND EFFECT ON SPEED PARAMETERS AND SUBSEQUENT PERFORMANCE.

F.ter Woort[1], G. Dubois[2], S. O'Connor[3], E. van Erck-Westergren[1]

[1] Equine Sports Medicine Practice, Waterloo, Belgium; [2] Arioneo, Paris, Île de France, France; [3] Hong Kong Jockey Club, Department of Veterinary Clinical Services, Sha Tin Equine Hospital, NT, Hong Kong.

Paroxysmal atrial fibrillation (pAF) occurs sporadically and has been implicated in poor performance. Currently, diagnosis in large scale studies is based on detecting AF on a post-exercising ECG, due to limitations in obtaining large amounts exercising ECGs. The objective of this study was to describe short bursts of pAF detected during exercise with an equine fitness tracker.

In this retrospective cross sectional study, records of exercising ECG consultations were evaluated to identify horses with pAF. The ECGs, training data and performance were evaluated.

One hundred exercising race-horse ECG consultations were evaluated. Eleven ECGs with pAF were identified in 10 horses. History was missing for 2 horses. Presenting complaints included poor performance (4), previous history of arrythmia (3), frequent isolated arrythmia at rest (1) and poor cardiac recovery (1). The pAF occurred in all horses during peak exercise (60-68 km/h, HR 206-217 bpm), with a normal ECG in warmup and recovery. PAF duration ranged from 10-90 seconds, returning to normal rhythm in all horses before the end of peak exercise (normal post-exercise ECG). During the pAF episode, 5 horses decelerated, 3 accelerated and 3 horses' speed was unaffected. Immediately after the pAF, 5 horses slowed down, 4 horses didn't change their speed and 2 horses accellerated. Unsurprisingly, RMSSD and SDNN during peak exercise were high in all horses. During recovery, RMSSD was normal and SDNN was high compared to published reference ranges. Racing performance following their pAF episode was available for 8 horses: 5 retired, 2 finished in the bottom 3rd of their subsequent 3 races and one horse never returned to racing.

In conclusion short bursts of pAF can be detected during training with a fitness tracker. These cases of pAF returned to a normal rhythm before the end of exercise and would be missed on a post-exercise ECG. Their immediate impact on speed appears variable in this small data set. However, none of the horses raced successfully after their episode of pAF.

P2

FROM DISCOVERY TO MARKET: MOVING EQUINE INNOVATION FORWARD

C.A. Cole[1]

[1]Technical Partner, Digitalis Ventures, 11 Times Square, Suite 1500A, Digitalis Ventures,11 Times Square, Suite 1500A, New York, NY 10036

There is often a wide gap between discovery at research institutions and the commercialization of those discoveries. While many institutions try to help with the process, their goals and priorities may not entirely align with those of the innovator. In addition, the motivation and skill set of an academic researcher is not necessarily that of an entrepreneur. Bringing innovations to the market in the equine industry also faces unique challenges. Compared to companion or production animals, the total addressable market for any equine product or service is relatively small. In addition, the equine world is fragmented. The racing industry is very different from the performance horse world, and this latter market fragments further into the various disciplines. Veterinarians are one of the few commonalities that span the entire equine segment. How can those of us dedicated to improving the health and welfare of the horse come together to advance the innovation process from discovery to new products and services in the market that address the needs of horses and their human connections. The goal of this session will be to review the opportunity from an investor perspective. What are various investor types looking for in an investment and how can start-ups in the equine space make themselves attractive to them.

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OPTIMIZING PERFORMANCE: FITNESS ASSESSMENT IN MARATHON DRIVING HORSES AND PONIES

Carolien Munsters [1,2], Esther Siegers[2], Marianne Sloet van Oldruitenborgh-Oosterbaan[2,3]

[1]Equine Integration, Hoogeloon, The Netherlands, [2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands, [3]Eikenlust Equine Consultancy, Bilthoven, The Netherlands

Driving is the oldest equestrian discipline, with combined driving consisting of three phases: dressage, marathon, and cones. At the highest level (3* for single and pair), horses complete 8 obstacles in the marathon phase, each within 40 – 60 seconds. Single horses, ponies, pairs, or teams of four navigate the obstacles at a canter, aiming to complete it fault-free in the shortest time. Between obstacles, brief recovery is possible, but an average speed of 3.6 – 3.9 m/s must be maintained. A wide variety of horses, ponies, and breeds participate, and yet no standardized method for assessing their fitness exists. The aim of the study was to evaluate the fitness of marathon driving horses and ponies using an exercise test specifically designed for driving.

Twenty-six carriage driving horses (n=12) and ponies (n=14) participated in one or two stepwise standardized exercise tests (SET) in the period between 2016 - 2024. Twenty-two horses and ponies were competing at the highest level (3*) and were in preparation for an elite event (selection for World Championships in single or pair driving). Two ponies performed at 2* level and two ponies at novice level. Data from 37 SETs were available for analysis.

All SETs were performed individually at the beginning of the competition season between February and April. Eleven horses (5 horses, 6 ponies) performed the SET again in the in the subsequent years (SET-2), average time period between SETs was 1.7 ± 1.1 years. At SET-1, horses and ponies were on average 11.0 ± 2.7 years old (range 7 to 17 years). The average height of the ponies was 140.2 ± 11.6 cm (range 115 to 148 cm) and of the horses 165.3 ± 3.7 cm (range 160 to 172 cm). The SETs consisted of a warm-up phase at walk for 5 - 10 minutes (at a speed of 1.7 ± 0.2 m/s), followed by two bouts of 2 x 400m at trot (speed horses 4.0 ± 0.5 m/s; speed ponies 3.6 ± 0.4 m/s). After the trotting phase, horses had to perform four steps of 2 x 400 m canter at increasing speeds, with 3 minutes of recovery at walk in between. During the SETs, animals were equipped with a commercially available heart rate monitor (HR; bpm) with integrated GPS (m/s). Ambient temperature (◦C) and relative humidity (RH, %) were measured during all SETs using a heat stress wet bulb globe temperature (WBGT) device.

Blood samples were collected by venipuncture of the left or right jugular vein to determine plasma lactate concentrations (LA, mmol/L). LA was determined after 60–90 s after after the first 1x 400m trot bout, after each of the four canter bouts and after 10 minutes of recovery at walk. If horses had a LA > 1.0 mmol/L (resting value) after the first 1 x 400m trot bout, LA was measured again after the second 400m trot bout. When LA was <1.0 mmol/l after the first trot bout, no extra LA measurement was performed after the second 400m at trot. When LA values were above 1.0 mmol/L after trot, and for the ponies smaller than 1.38 cm, LA was measured after the first 400m of the first canter step before proceeding to the next 400m canter. SETs were stopped if LA reached results of 6.0 mmol/L or higher. If horses reached LA results > 6.0 mmol/L after the first or second canter bout, the HR, LA and speed data of the preceding trot bouts were included in the analysis to calculate fitness parameters. From the HR and LA data obtained, the following fitness parameters were calculated: HR at LA of 2 or 4 mmol/L (HR_{LA4} and HR_{LA2}, respectively) and speed at LA of 2 or 4 mmol/L (V_{LA2} and V_{LA4} respectively). Not all horses and ponies were able to complete the entire SET.

To check whether the normality assumption was reasonable, normality probability plots of the residuals were made. If the normality assumption did not hold, the data were log-transformed to obtain a normal distribution. HR, LA and speed data were analyzed using a linear mixed-effect model with horse as a random effect and SET, animal type (horse or pony) and SET*animal type interaction as fixed effects. Akaike's Information Criterion (AIC) was used for model reduction. The model for which there was no further reduction possible was taken as the final model, and the remaining factors were considered important. The 95% likelihood confidence intervals (95% CI) were reported for the important effects. Chi-square tests were used to evaluate the difference between horses and ponies and bouts performed at trot or canter, where significance was set at $p<0.05$.

During the SETs, average Web Bulb Globe Temperature (WBGT) was 7.3 ± 1.5°C with a relative humidity (RH) of 54.6 ± 17.4% and ambient temperature of 9.8 ± 1.6°C, there was no difference between SETs. Most SETs (n=34) were conducted on a firm 400 m grass track, while three SETs (all SET-,1 one horse and two ponies) were performed on an 800 m sand track. The speeds for ponies during the four SET bouts were 3.7 ± 0.4, 4.4 ± 0.9, 5.3 ± 0.8 and 6.2 ± 0.7 m/s; and for horses 5.1 ± 0.9, 5.8 ± 0.8, 6.6 ± 0.7 and 7.4 ± 0.9 m/s. There was no significant difference in the speeds between SET-1 and SET-2.

During SET-1, significantly more ponies (n=13, $p<0.001$) than horses (n=3) could only perform 3 bouts of canter before reaching LA > 6.0 mmol/L and the last trot bout needed to be included in the analysis. In SET-2, both horses and ponies completed the same number of steps before reaching LA > 6.0 mmol/L. HR_{LA4}, HR_{LA2} and V_{LA2} did not differ between horses and ponies, but V_{LA4} was significantly higher in horses compared to ponies (estimate 0.672, 95% CI, 0.244, 2.160). For all animals, V_{LA4}, V_{LA2} and HR_{VLA4} were significantly lower in SET-1 compared to SET-2 (estimate; -0.692, 95% CI -1.022, -0.362; estimate; -0.690, 95% CI -1.082, -0.323 and estimate; -6.301, 95% CI -10.290, -2.313, respectively; Table 1).

In SET-1, 3 horses and 4 ponies they reached their V_{LA4} at their first canter bout (working canter speed). Two ponies were performing at novice level, but the other 5 animals were expected to perform at high competition level soon after the exercise test. In SET-2, only one pony (novice level) reached his V_{LA4} at their first canter bout. Animals reaching LA levels of 4 mmol/L or higher during the first canter bout at working speed, which is lower than the competition speeds, seemed to be unfit for the physical demands asked at competition.

Table 1. The fitness parameters heart rate at lactate concentration of 4 or 2 mmol/L (HR_{LA4} and HR_{LA2}, respectively) and speed at lactate concentration of 4 or 2 mmol/L (V_{LA2} and V_{LA4} respectively) calculated from marathon driving horses and ponies participating at one or two standardized exercise tests (SET-1 and SET-2).

	SET-1		SET-2	
	Ponies (n=14)	**Horses** (n=12)	**Ponies** (n=6)	**Horses** (n=5)
HR_{LA4}	177 ± 11*	179 ± 13*	182 ± 8	184 ± 12
HR_{LA2}	160 ± 15	164 ± 14	166 ± 10	168 ± 15
V_{LA4}	5.4 ± 1.3*	6.9 ± 0.9**	5.6 ± 1.7	7.0 ± 0.9**
V_{LA2}	4.5 ± 1.2*	5.8 ± 0.8*	4.7 ± 1.7	6.0 ± 0.7

* Indicate a significant difference between SET-1 and SET-2 based on Aika's information criterium (AIC), ** Indicate a significant difference between horses and ponies based on Aika's information criterium (AIC).

In conclusion, the study highlights clear differences in lactate accumulation patterns between horses and ponies during SETs. In SET-1, significantly more ponies than horses reached higher lactate levels (LA > 4.0 mmol/L) early, suggesting lower initial fitness, though this difference disappeared in SET-2. Horses exhibited higher V_{LA4} values than ponies, likely due to their greater body size and proportionally longer limbs, which biomechanically facilitate higher locomotor speeds. Across all animals, SET-2 results showed improved fitness parameters compared to SET-1, reflecting adaptations to training and increased conditioning over time.

The variation in SET design proved necessary due to the diverse fitness levels and physical characteristics of the horses and ponies, particularly as switching from trot to even slow dressage canter represents a significant intensity step for some of the animals. Notably, all animals in this study were used for marathon driving, a discipline requiring sustained canter at high intensity with frequent turning, pulling, and acceleration at canter in obstacles. The results emphasize the importance of training canter at high intensity to meet the fitness demands of this discipline. Animals reaching V_{LA4} during their first canter bout were predominantly unfit for competitive demands, as the speed at this step was lower than competition demands, highlighting the need for tailored assessments to optimize training and performance for both horses and ponies. This reinforces the importance of monitoring heart rates and lactate parameters in equine athletes to optimize training and performance outcomes.

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A PILOT STUDY OF MOVEMENT ASYMMETRY IN FEI-LEVEL SPORT HORSES: PREVALENCE AND CORRELATION TO PERFORMANCE

Erin K Contino[1], Jodie Daglish[2], Chris E Kawcak[1]

[1]Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO USA, [2]Newmarket Equine Hospital, Newmarket, Suffolk, UK

Lameness and musculoskeletal pain are leading causes of lost training and competition days in both equine and human athletes. Additionally, lameness in horses is generally under-detected—a majority of horses that are considered sound by their owners or trainers are lame when evaluated subjectively by veterinary specialists or by objective measures. Yet the concept of a "serviceably sound" horse is well recognized in the equine industry, suggesting that horses may still perform their job adequately, or even well, in the face of mild lameness or movement asymmetry. However, the relationship between lameness and performance is not well understood. Furthermore, increasing discussions surrounding horse sports' social license to operate muddies the water when considering that horses are likely competing, and even succeeding, in the face of musculoskeletal pain and/or lameness. Thus, this pilot study aimed to 1) determine the prevalence and magnitude of lameness or asymmetry in equine athletes competing at the FEI (Federation Equestrian International) level in various disciplines; and 2) to assess the impact of lameness on performance outcomes. We hypothesized that 1) the majority of horses competing at the FEI level across disciplines will have subtle lameness; 2) that subtle lameness will not directly correlate to performance outcome; 3) that more severe lameness will negatively affect horses' performance outcome; and 4) that outcomes of objectively scored disciplines will be less influenced by lameness than subjectively scored disciplines.

Horses competing at FEI sanctioned competitions (and thus competing under FEI 'Clean Sport' rules) in Eventing, Show Jumping or Dressage were eligible for inclusion. A single venue for each discipline was chosen based on timing and show management cooperation from 2017-2018 and included The Event at Rebecca Farm, Tryon International Equestrian Center and the Adequan® Global Dressage Festival. Competitors were educated on the concept and details of study and could voluntarily enroll their horse(s).

Enrolled horses were evaluated in hand at trot on firm ground in a straight line. Asymmetry was evaluated subjectively on a 0 (no lameness) to 5 (non-weight bearing) scale consistent with the AAEP lameness scale, by consensus of 2 experienced lameness specialists. Objective measurements were acquired simultaneously with a commercially-available inertial measurement system (Lameness Locator, Equinosis, Columbia, MO) that reports head and pelvis asymmetry as a vector sum (Q score); larger Q scores correlate with higher degree of asymmetry. All evaluations were performed at, but prior to the start of, the competition. All results were maintained confidentially from competitors and officials as to not influence the competitor or competition in any way. The results of the objective lameness examination were available to competitors *after* the completion of the competition, upon request.

Competition results were obtained through the competition database and consisted of the final raw score, finishing place (rank), and finishing percentile. A subjective lameness score for each horse was assigned based on the lamest limb lameness grade and a cumulative lameness score was calculated by summing the lameness grades of all four limbs. For objective assessment, Q scores were calculated in a similar fashion. Summary statistics were calculated for horse demographics and magnitude and prevalence of single- and multi-limb lameness. A chi-squared test was used to compare raw competition scores to the lamest limb and cumulative lameness score. Spearman's rank and Pearson correlations were performed to test correlation between lameness grade and finishing score, finishing rank, and finishing percentile.

Of 299 eligible horses at the three different competitions, 84 (28%) were voluntarily enrolled by their owners. Enrollment rate was highest among Show Jumpers (22/38; 58%) and lowest among Dressage competitors (15/125; 12%); thirty-five percent (47/136) of Eventers enrolled. The majority of enrolled horses were Warmbloods (54), followed by Thoroughbreds (13), Irish Sport Horses (11) and crossbreds (6). The average age of enrolled horses was 11.6 years (range 6-20 years).

Subjectively, asymmetry was identified in 85% of Eventers, 91% of Show Jumpers and 100% of Dressage horses which represents 89% of enrolled horses. On subjective assessment, asymmetry in multiple limbs was recorded in 44% of horses (51% of Eventers, 32% of Show Jumpers and 40% of Dressage horses). Similarly, when evaluated objectively, 88% of horses showed some degree of asymmetry including 91% of Eventers, 77% of Show Jumpers and 93% of Dressage horses. Asymmetry in multiple limbs was appreciated by objective measurement in 51% of the study population (54% of Eventers, 36% of Show Jumpers and 60% of Dressage horses). The magnitude of the asymmetry was mild with a mean and median subjective lamest limb score of 1.0 (range 0 to 3.5). Objective mean and median Q scores were 11.0 and 10.9 (range 0 to 43.9), respectively. Similarly, the mean and median subjective cumulative lameness scores were also mild (1.3 and 1.0, respectively; range 0 to 5). The objective cumulative lameness score ranged from 0 to 50 with a mean of 14.9 and median of 13.6.

The sample population encompassed horses that finished in the top (28.6%), middle (35.7%) and bottom (20.2%) thirds of their divisions and 15.4% that did not finish. For dressage horses, there was no significant association between lameness and performance outcomes however, there was a trend ($p=0.08$) towards a moderate correlation (Pearson $r= 0.46$) between increasing objective cumulative lameness scores and an increasing (worse) final placing. For eventing horses, there was no correlation between any lameness parameters and overall competition results, nor to dressage, cross-country or jumping phase results. In show jumping horses there was a trend for a weak correlation (Spearman $r=0.37$; $p=0.09$) between increasing objectively graded lameness parameters and improved final percentile but these did not reach statistical significance.

Prevalence of lameness in this subset of horses was high, with nearly 90% of enrolled horses showing some degree of lameness or asymmetry. This is greater than levels of 'incidental lameness' reported elsewhere.[1,2] Horses in this study were competing under FEI Clean Sport rules, thus were not under the influence of anti-inflammatory or other pain modulating medications which may have resulted in a higher prevalence of lameness compared to horses in the general sporting population. This study relied on voluntary participation which may have resulted in sign-up bias. Competitors with a concern about their horses' soundness may have been less likely to enroll for fear of a lameness being detected. Conversely, these same competitors may have preferentially enrolled their horse in order to access the objective lameness examination report following competition. Either scenario could have skewed the results.

Despite the high prevalence of lameness, it was not surprising that lameness in this population tended to be mild. This study was unlikely to include any overtly or severely lame horses as such horses would likely be withdrawn prior to presenting to an FEI jog. Perhaps most importantly, the subjective and objective assessments were ultimately a measure of asymmetry which is not always caused by musculoskeletal pain. Thus, asymmetry cannot be used interchangeably with lameness. It is possible that a proportion of these horses were not experiencing musculoskeletal pain despite having movement asymmetry. Regardless, there were no statistically significant correlations between any of the lameness scores and competition results. This supports the concept of the 'serviceably sound horse' that can successfully compete in the face of low-grade single- or multi-limb lameness or asymmetry.

This study failed to determine a degree of lameness that negatively influenced performance. This is most likely due to the lack of enrolled horses with more pronounced lameness as well as the small sample size. Additionally, it is possible that horses with more lameness may not be performing adequately enough in training to be enrolled in competition. There was a trend for lamer dressage horses to not perform as well as, and conversely for lamer show jumping horses to perform better than, their counterparts. This may suggest that lameness has a greater impact on subjectively, compared to objectively, scored competitions. This aligns with a recent study that demonstrated that higher ridden pain ethogram scores were negatively correlated with judges scores in dressage competition.[3]

In conclusion, mild single- and multi-limb lameness is very common in FEI-level horses and does not appear to be significantly performance limiting. However, this does not necessarily make it acceptable from a social licensing standpoint, especially if it represents a true lameness versus a non-painful asymmetry. Measuring the psychological aspects of pain and performance in equine athletes is difficult and this study underscores the need for better methods to evaluate a horse's fitness to compete. Recent work has demonstrated a correlation between the ridden horse ethogram and competition outcome and it seems likely that this may be a more accurate way to measure a horse's suitability and readiness to compete compared to lameness examinations alone.[4]

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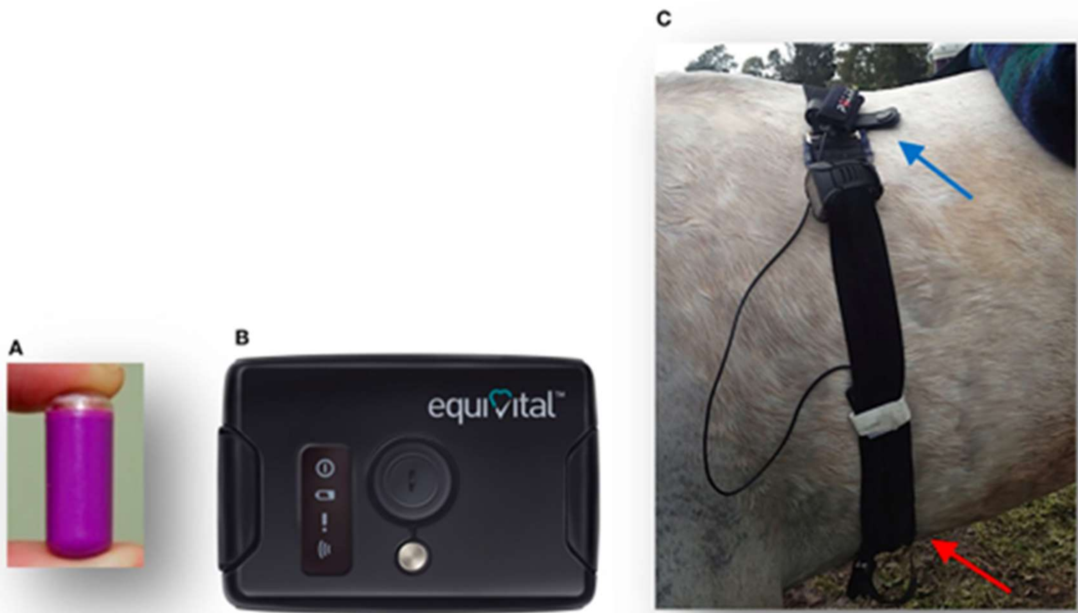
ASPECTS OF CONTINUOUS MONITORING OF SKIN SURFACE TEMPERATURE IN ENDURANCE HORSES DURING FIELD EXERCISE

Elisabeth-Lidwien J.M.M. Verdegaal[1,2*], Gordon S. Howarth[1], Todd J. McWhorter[1] and Catherine J.G. Delesalle[2].

[1]Thermoregulation Research Group, School of Animal and Veterinary Sciences, Roseworthy Campus, University of Adelaide, South Australia, Australia; [2]Research Group of Comparative Physiology, Department of Virology, Parasitology & Immunology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

A progressive increase in the prevalence of heat stress events and exertional heat illness (EHI) casualties is anticipated due to global warming. Of equal concern is increasing evidence that EHI is underreported, and only the more severe and overt clinical cases are being recognized, leaving many mild cases unnoticed and undocumented. Improved monitoring is critical to safeguard thermoregulatory wellbeing in humans and a wide range of animal athletes including horses. Therefore, research groups worldwide are investigating the accuracy and reliability of invasive and non-invasive devices that may add to the early detection of risk for the development of EHI under different exercise and climatic conditions. We have previously reported on the reliability of using a telemetric gastrointestinal (GI) pill (Verdegaal et al., 2021) to continuously monitoring the core body thermal response in endurance and trotter horses in the field.
One practical and non-invasive approach to measure the thermoregulatory response is the continuous monitoring of surface skin temperature (Tsk). Within the rapidly expanding wearable industry, surface Tsk monitoring devices are constantly being upgraded to facilitate collection of high-resolution datasets. However, one critical question remains: how should we interpret that data? The current study is the first to compare simultaneous measurements of Tsk with core body temperature (Tc) using a GI pill during 40 km endurance loops in real-life field competitions. For this purpose, large datasets of Tsk and Tc recordings were analyzed and compared. As a result, an integrative real-time view was obtained on how the equine body copes with metabolic heat (MH) production and how the Tsk responded during exercise in the field.
The Tsk and Tc profiles were established during endurance exercise and continued during recovery. For this purpose, 13 endurance horses were equipped with four wearable non-invasive monitoring devices (GI pill, Tsk device (Figure 1), heart rate [HR] monitor and global positioning system [GPS] receiver).

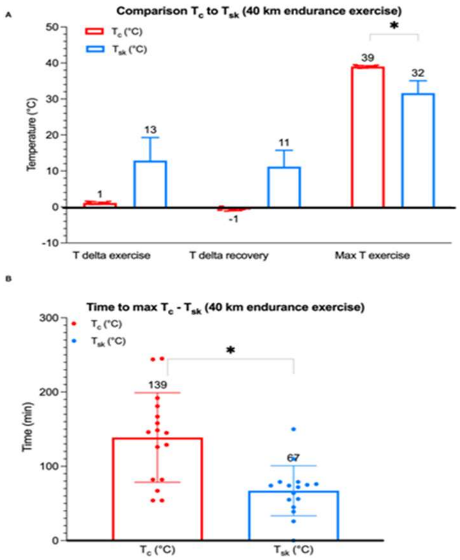
Fig. 1. T_{sk} and T_c monitoring equipment: gastrointestinal temperature pill (A); an external receiver Equivital® Sensor Electronics Module (SEM) with an infrared sensor to measure T_{sk} (B); a modified belt; red pointer, SEM device & T_{sk} thermistor position; blue pointer, GPS device.



In addition, on each data collection day, ambient temperature (Ta) data were obtained from the Australian Bureau of Meteorology. Recordings performed during each 40 km loop exercise period included Tc , Tsk, speed and HR. A temperature-time profile curve of Tsk and Tc for each individual horse was constructed for each exercise and recovery period. The net area under the curve (AUC, baseline set at rest Tsk) was calculated using the trapezoidal method of Tsk over time (minutes) expressed as °C x minutes.

Statistical analyses included comparison and correlation analyses with different approaches to evaluate the potential of the Tsk data as a reliable proxy to assess the core body thermoregulatory response. The relationship between Tsk and Tc was assessed using scatterplots (8 horses each performing two subsequent 40 km loops). In addition, maximum Tsk and Tc and the time to reach maximum Tsk and Tc were compared using paired t-tests. Delta Tsk during exercise and recovery periods were compared. The association between Tsk and Tc at different points in time and the association with HR or coat color were analyzed using a general linear model ANOVA. Statistical significance was set at $\alpha = 0.05$.

All horses completed their exercise trials without any adverse events. No horses were withdrawn by owners. On all occasions, the Ta was relatively cool: mean minimum 6.7 ± 0.4°C, mean maximum 18.4 ± 2.9°C. One of the most significant findings of the current study was the substantial individual variability in the recorded Tsk responses, emphasizing the importance of individual monitoring of exercising horses. Equally importantly, the present study also demonstrated no direct correlation between the Tsk and Tc time profiles (Figure 2).



An interesting finding of the current study was the association of a greater Tsk at the end of exercise with a significantly lesser Tc at the end of recovery (60 minutes). Several hypotheses could be considered to explain this pattern: Firstly, the raised Tsk could indicate the launch of an active thermoregulatory response to anticipate the increased Tc and once the MH is successfully dissipated, the Tc decreases. This argument can be coupled with the effect of cooling post-exercise, which may be more prominent when Tsk is greater and ultimately results in higher dissipation of MH and a reduced Tc.

The lack of correlation between Tsk and Tc time profiles emphasizes that horse owners should not rely on Tsk when monitoring exercising horses in a cool environment. On all occasions, peak Tc values were significantly higher than peak Tsk values, and on all occasions, there was a significantly decreased time to peak Tsk than to peak Tc. No significant difference could be identified between delta Tsk when comparing exercise and recovery periods. It is therefore questionable whether continuous monitoring of Tsk is a reliable proxy to monitor the thermodynamic response in exercising horses.

Considering that additional factors such as air movement and sweating can easily and quickly change Tsk without directly affecting Tc, further research is needed into reliable monitoring methods.

Another study by our research group reviewed Tsk monitoring and concluded that Tsk is primarily used as a non-invasive tool to assess the thermoregulatory response pre- and post-exercise, particularly employing infrared thermographic equipment (Verdegaal et al. 2023). However, only a few studies have used thermography to monitor skin temperature continuously during exercise. Our review (Verdegaal et al. 2023) highlighted that, while monitoring Tsk is straightforward, continuous Tsk alone does not always reliably estimate Tc evolvement during field exercise. In addition, inter-individual differences in thermoregulation need to be recognized and accounted for to optimize individual wellbeing.

The ongoing development of advanced wearable monitoring technology and data modeling will facilitate timely intervention in horses at hyperthermic risk, improving their welfare. However, at this point, infrared thermographic assessment of Tsk should always be used in conjunction with other clinical assessments and veterinary examinations for a reliable monitoring of horse welfare.

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INTRAMUSCULAR TEMPERATURE SENSORS FOR MANAGING POST-EXERCISE COOLING IN ATHLETIC HORSES

Michael S. Davis[1], Joe D. Pagan[2], and Ryon W. Springer[2]

[1]*Waypoint Veterinary Education and Consulting LLC, Edmond, Oklahoma, USA;* [2]*Kentucky Equine Research, Versailles, Kentucky, USA*

Skeletal muscle hyperthermia is a virtually inevitable consequence of athletic performance due to the exothermic nature of the energy pathways that power muscle contraction. Although the increased rate of heat production that occurs during exercise is partially mitigated by a concurrent increase in whole body heat transport and dissipation, accumulation of body heat, particularly in the working muscles where the heat is generated, can be a substantial cause of not only exercise fatigue but also exercise-induced heat injury. Thus, there is a long-standing and increasing interest in improving heat dissipation during and after exercise.

Recent advances in microchip technology have paired the identification function of the chips with a passive thermistor sensor that will report temperature of the tissue in which the chip is placed when read with the chip reader. Implantation of a chip in a locomotory muscle allows rapid, accurate, and repeated measurement of muscle temperature both during exercise and during the post-exercise cooling period and is compatible with any active cooling process. Heat dissipation is a passive process that is governed by the heat gradient between the subject and its environment and is approximated by nonlinear 1st order kinetics with asymptotic approximation of pre-exercise (resting) body temperature. By facilitating the frequent measurement of temperature during the cooling process, the implanted microchips can permit accurate quantification of this nonlinear process and provide a single measurement – cooling half-life or $t_{1/2}$ – that can be used to compare cooling rates under different experimental conditions.

All studies used horses at Kentucky Equine Research facilities in Kentucky and Florida that were implanted with percutaneous thermal sensing microchips (PTSM) in the middle gluteal muscle, exercised under a variety of different conditions (treadmill and track), then serial muscle temperature measurements were recorded for 1 hr during a variety of cooling interventions commonly used or recommended as management practices of equine athletes. All studies were randomized complete crossover design. Rate of gluteal muscle cooling was determined using both simple linear regression of time vs DeltaTemp (the difference between the post-exercise muscle temperature measurements and the pre-exercise muscle temperature measured immediately before the exercise session) as well as exponential curve-fitting of time vs DeltaTemp. Using this approach, the slope of the time (x-axis) and the natural logarithm of the DeltaTemp (y-axis) is converted to half-life ($t_{1/2}$) using the formula $t_{1/2} = -0.693/\text{Slope}$. The curve-fitting function in the statistical program (Prism 10.4.1) was further directed to apply a plateau constraint value of 0 to the process to optimize the resulting fitted curve and simulate complete return to normal body temperature. An advantage of characterizing the rate of cooling is that $t_{1/2}$ is independent of the magnitude of heating. Thus, variation in peak muscle temperature at the start of the post-exercise cooling period due to differences in the magnitude of exercise-induced hyperthermia had a minimal impact on the rate of cooling. The quality of the respective models of gluteal muscle cooling was determined by comparing the R^2 values from the linear and exponential regressions using a paired student's t-test. $T_{1/2}$ was analyzed using repeated measures one-way ANOVA (paired student's t-test when the study included only 2 groups), with $p<0.05$ considered statistically significant.

The exponential model of gluteal muscle cooling yielded superior R^2 values compared to the linear models (Exponential R^2 0.95±0.07, Linear R^2 0.85±0.13, $p < 0.0001$). Therefore, only the exponential rate of cooling constant ($t_{1/2}$) will be presented to describe the differences in cooling methods.

- Study #1 used 8 horses to compare Active cooling (a combination of hosing, misting, and application of cooling mats) to Passive cooling (walking and standing) following 14 minutes of treadmill exercise in hot-humid conditions. Active cooling ($t_{1/2}$ 20.73±7.65 minutes) was faster than Passive cooling ($t_{1/2}$ 53.55±38.59 minutes) ($p = 0.023$).
- Study #2 used 8 horses to determine the effect of hosing in hot dry conditions following 13 minutes of treadmill exercise. Hosing resulted in faster cooling compared to passive cooling (Hosing $t_{1/2}$ 12.74±5.78 minutes, Passive cooling $t_{1/2}$ 22.57±9.93 minutes, $p = 0.001$); however, it is important to

note that passive cooling was much faster in this study done under hot dry conditions compared to Study #1 performed in hot humid conditions where the Passive $t_{1/2}$ was more than double that in Study #2.

- Study #3 used 8 horses to compare the effects of 15 minutes of misting application of water vs 5 minutes of hose application of water following 2400 m of racetrack exercise (including 1000 m gallop and 200 m breeze). Hosing resulted in slightly faster cooling than misting (Hosing $t_{1/2}$ 10.28±3.90 minutes, Misting $t_{1/2}$ 14.11±7.70 minutes, p = 0.047).
- Study #4 used 8 horses to compare the effects of walking, application of water using a sponge, and hosing following 13 minutes of treadmill exercise in cool, humid conditions. Both hosing and sponging resulted in faster cooling than walking, but there was not a difference between hosing and sponging (Walk $t_{1/2}$ 33.22±11.10 minutes, Sponging $t_{1/2}$ 16.00±5.52 minutes, Hosing $t_{1/2}$ 16.57±3.10 minutes, p = 0.0002).
- Study #5 used 7 horses to compare the effects of hosing and allowing the water to remain on the horse vs hosing then scraping the water off the horse following 2400 m of racetrack exercise (including 1000 m gallop and 200 m breeze). There was no difference in the exponential rate of gluteal muscle cooling between scraping vs leaving the water on the horse (Scrape $t_{1/2}$ 10.66±3.82 minutes, Leaving $t_{1/2}$ 11.29±5.52 minutes, p = 0.270). Like Study #3, this study was conducted in a hot, humid environment in which evaporation is expected to play less of a role in heat dissipation, and thus the primary mechanism of cooling is likely the direct conduction of body heat to the cooler water that is applied during hosing.
- Finally, Study #6 used 7 horses to compare the effects of hosing the entire body vs just hosing the head and neck area following 2400 m of racetrack exercise (including 1000 m gallop and 200 m breeze). Hosing the entire horse resulted in much more rapid cooling of the gluteal muscles than hosing just the head and neck area (Whole $t_{1/2}$ 10.90±4.31 minutes, Neck $t_{1/2}$ 17.49±4.04 minutes, p = 0.003). Although concentrating the hose application of water on the head and neck has been advocated to help protect the horse's brain from the deleterious effects of hyperthermia, if this approach is used exclusively it occurs at the expense of muscle cooling.

The results of these studies illustrate the advantages of using PTSM placed in metabolically important locations instead of relying on rectal temperatures which only directly measure the temperature of the anal sphincter. Recent research has shown that under conditions of hyperthermia, skeletal muscle produces 2-3X greater amounts of reactive oxygen species per unit of oxygen consumption than at normal temperature. Thus, the longer the muscle is at an elevated temperature, the more oxidative damage likely is being suffered. By directly monitoring the skeletal muscle temperature, the horse can be effectively cooled and potentially reduce post-exercise muscle damage. Furthermore, the use of a device that easily allows frequent measurements will allow the user to gain a greater understanding of the rate of cooling and the active measures that are most effective in expediting post-exercise muscle cooling.

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EFFECTS OF BLOOD FLOW RESTRICTION TRAINING ON NORMAL EQUINE CARTILAGE AND SUBCHONDRAL BONE

Sherry A. Johnson[1], Melissa R. King[2], Christopher E. Kawcak[2], Kurt T. Selberg[3], David D. Frisbie[2]

[1]Equine Sports Medicine, LLC Pilot Point, TX, USA; [2]Colorado State University, Department of Clinical Sciences, Fort Collins, CO, USA; [3]Colorado State University, Environmental & Radiological Health Sciences Dept, Fort Collins, CO, USA.

Improving muscular strength in absence of high-intensity exercise through the use of blood flow restriction (BFR) training has become a key rehabilitative modality utilized by human physical therapists to restore muscle strength following arthroscopy. Specifically, this low-intensity training promotes a faster return to physical function and less co-morbidity in human patients by mitigating arthrogenic muscle inhibition [1-2] that would otherwise delay or prohibit full return to orthopedic function. Subsequently, BFR has become a post-operative modality for human knee osteoarthritis [3-4]. Additionally, the downstream effects BFR may have on osseous remodeling is a source of ongoing investigation [5], and of particular interest to those clinicians incorporating its use into post-fracture repair rehabilitation. Despite BFR's increasingly widespread use, its effects specifically on articular cartilage and subchondral bone properties are unknown.

Equine-specific BFR research and clinical usage remains in its infancy. Despite initial safety validation and tendon-specific equine BFR investigations [6-7], BFR's effects on equine articular cartilage and subchondral bone are still unknown yet remain imperative to understanding its potential appropriate orthopedic applications in horses. Therefore, the overarching goal of this project was to determine the effects of BFR specifically on normal equine articular cartilage and bone as safety validation for equine use and further human translational investigation.

This study was a controlled prospective experiment. All animal work was approved by IACUC at Colorado State University (Protocol #1013). Four forelimbs of four horses were exposed to 40 BFR-walk sessions (10-minute interval walking) on a treadmill over a 56-day study period with their contralateral forelimbs serving as untreated controls as previously reported [6-7]. Similarly, four forelimbs of four control horses were exposed to 40 sham cuff walk sessions. On study day 56, all horses (n = 8) were humanely euthanized. Following euthanasia, both forelimbs of all horses were harvested at the level of the scapulohumeral joint and a post-mortem computed tomographic (CT) evaluation was performed (Siemens Somatome Definition AS 64). To evaluate bone density, a region of interest (ROI) 4mm distal to the articular surface was drawn around the entire circumference of the third carpal bone using the free-hand ROI tool. Hounsfield Units (HU) readings of each third carpal bone were then recorded. All ROI's and subsequent HU readings were determined by a board-certified veterinary radiologist blinded to treatment groups. Following CT evaluation, soft tissues were removed and gross lesions of all third carpal bones were recorded and photographed. Osteochondral samples from the third carpal bones were harvested, paraffin embedded and stained with H&E for future histologic assessment.

Following macroscopic evaluation as previously described [8], articular cartilage sections (5mm biopsies) were collected from each third carpal bone [9] and processed for routine histologic analysis (hematoxylin and eosin (H&E) and safranin O fast green (SOFG)), then macroscopically and microscopically graded as previously described [8]. Osteochondral fragments measuring 3mm in diameter were also harvested from the third carpal bone and analyzed for live cells and glycosaminoglycan (GAG) content from sites previously described [9]. The live/dead viability was determined using the live/dead staining reagent[2]. The total content of sulfated GAG was determined by 1,9 dimethylmethylene blue (DMMB) binding as described previously [9-11].

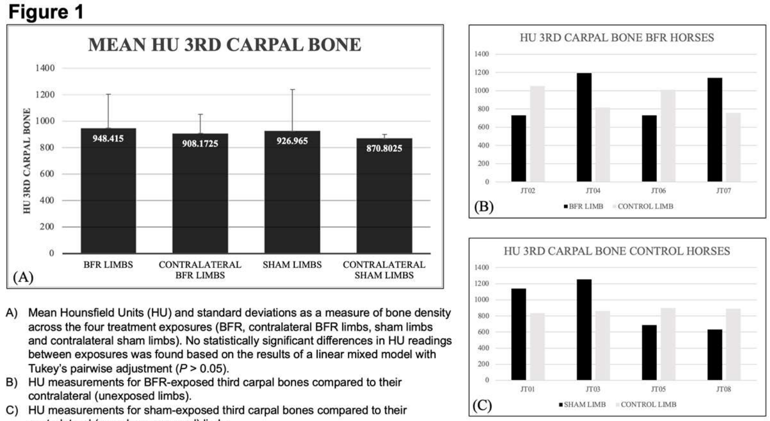
To investigate the relationships between BFR exposure and response variable of HU, a linear mixed model was fit. The model included fixed effect of treatment and random effect of horse. To investigate the relationships between BFR exposure and response variables of live cell viability percentage and GAG

[2] Thermo Fisher Scientific, Waltham, MA, Catalog #L3224

levels, a linear mixed model was fit. The model included fixed effects of treatment and random effect of horse. Diagnostic plots were used to assess assumptions of equal variance and normality. Based on these plots, assumptions were met. Comparisons across exposure were conducted utilizing the lmer4 and emmeans packages. Tukey's adjustment of *P*-values for pairwise comparisons were used.

No statistically significant differences in HU readings between treatment groups were found ($P > 0.05$, **Figure 1**). Qualitative differences between treatment groups for macroscopically and microscopically assessed articular cartilage criteria were not appreciated between treatment groups. No statistically significant differences in any evaluated GAG or live cell viability values between treatment groups were appreciated ($P > 0.05$, **Figure 2**).

Figure 1



A) Mean Hounsfield Units (HU) and standard deviations as a measure of bone density across the four treatment exposures (BFR, contralateral BFR limbs, sham limbs and contralateral sham limbs). No statistically significant differences in HU readings between exposures was found based on the results of a linear mixed model with Tukey's pairwise adjustment ($P > 0.05$).
B) HU measurements for BFR-exposed third carpal bones compared to their contralateral (unexposed limbs).
C) HU measurements for sham-exposed third carpal bones compared to their contralateral (non sham-exposed) limbs.

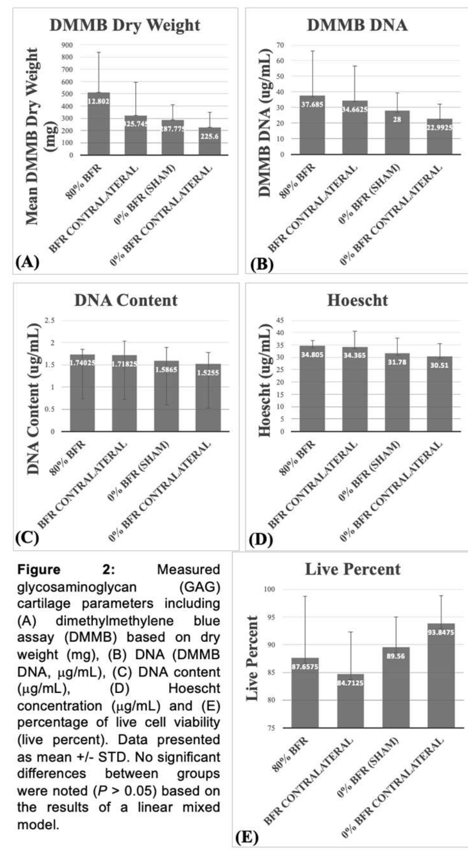


Figure 2: Measured glycosaminoglycan (GAG) cartilage parameters including (A) dimethylmethylene blue assay (DMMB) based on dry weight (mg), (B) DNA (DMMB DNA, µg/mL), (C) DNA content (µg/mL), (D) Hoescht concentration (µg/mL) and (E) percentage of live cell viability (live percent). Data presented as mean +/- STD. No significant differences between groups were noted ($P > 0.05$) based on the results of a linear mixed model.

The results of this investigation suggest that no statistically significant changes in (normal) bone density as assessed on post-mortem CT evaluation occurred as a result of exposure to 40 BFR walking sessions. With a small study population and wide standard deviations, it is not surprising that statistically significant differences were not appreciated. It was interesting to note that despite the lack of significant differences in HU readings between BFR and sham-exposed limbs, the paired forelimbs of all horses consistently demonstrated appreciable differences that seemingly were unrelated to BFR or sham cuff application. The reason for this remains unknown, but a possible explanation is that horses may have inherent physiologic variation related to handed-ness that manifests itself as preferential loading of one forelimb over the other. Baseline CT evaluation would have helped identify if these left to right differences existed prior to any BFR or exercise exposure had it been monetarily feasible.

Additionally, no deleterious effects of BFR walking exercise on articular cartilage based on morphologic, live cell viability and biochemical methods were appreciated. While widespread safety conclusions cannot be extrapolated, these results are encouraging and represent the first report of cellular-level effects of BFR on articular cartilage, specifically that of the joint immediately distal to the site of cuff application. Realization of BFR's physiologic effects on articular cartilage are currently unknown, but would help guide its safe orthopedic use in humans and horses. Limitations of this study include a small study population evaluating BFR's effects in only healthy articular cartilage and bone without analysis of synovial fluid, cartilage biomechanics or bone histomorphology. Further investigations incorporating more robust tissue analysis in osteoarthritic cartilage and/or various experimental injury models represent logical next investigative steps.

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EFFECT OF DIFFERENT COOLING METHODS ON MUSCLE TEMPERATURE RECOVERY IN EXERCISED TWO-YEAR-OLD THOROUGHBRED RACEHORSES

J. D. Pagan[1], R. W. Springer[1], E. D. Robyn[1], I. A. Robinson[1] and M. S. Davis[2]

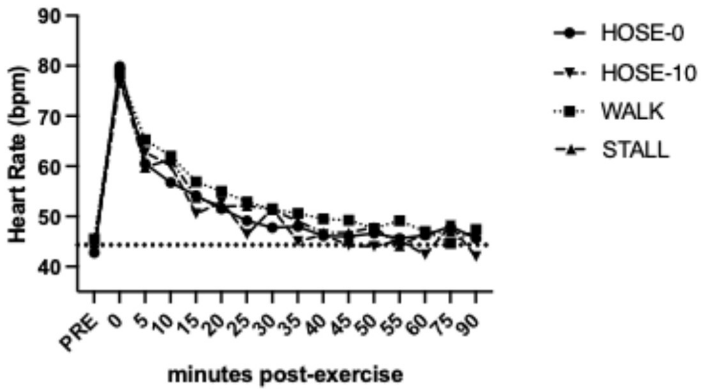
[1]Kentucky Equine Research, Versailles, Kentucky, USA; [2]Waypoint Veterinary Education and Consulting LLC, Edmond, Oklahoma, USA

Most studies evaluating post-exercise cooling of horses under field conditions have relied on rectal temperature as an indirect measure of core temperature. Direct measures of core and muscle temperature have been confined to treadmill exercise because of the instrumentation needed to make these measurements. Recently, percutaneous thermal sensing microchips (PTSM) have been introduced as more practical alternatives for routine muscle temperature monitoring during and after exercise (Kang et al 2022). Temperatures measured with PTSM placed in the gluteal and pectoral muscles of horses have been shown to have a high positive correlation with central venous temperature during and after treadmill exercise (Kang et al, 2020). Racehorse trainers often use hand-walking and hosing with tap water as a means of cooling horses after exercise. The objective of this study was to investigate the impacts of field-based cooling practices on muscle temperature recovery in two-year-old Thoroughbred racehorses undergoing routine training gallops.

Eight two-year-old Thoroughbred geldings in early race training previously implanted with middle gluteal muscle (GLUT) PTSM were used in a duplicated 4 x 4 Latin Square design over four consecutive weeks. The study was conducted in April and May at Kentucky Equine Research's Performance Center in Ocala, Florida. Horses were blocked into their respective replication of the Latin Square using peak GLUT temperatures obtained from previous exercise. Horses were exercised on a 1200 m dirt-sand racetrack four to five days each week. The last day of each week, horses galloped 1200 m (9 to 10 m/s) with a 400-m jog (3.5 to 4.0 m/s) and 200-m walk (1.4 to 1.5 m/s) as a warm-up and cool-down exercise (total: 2400 m). After exercise, horses underwent one of four cooling treatments: hosing with tap water for 5 min then hand-walking for 10 min (HOSE-0), hand-walking for 10 min then hosing with tap water for 5 min (HOSE-10), hand-walking for 15 min (WALK), or standing in a stall for 15 min with a fan overhead (STALL). All horses stood in stalls equipped with fans from 15 to 90 minutes post-exercise with access to fresh water and timothy hay. Heart rate (HR), respiratory rate (RR) and GLUT temperature were measured before and at 5-minute intervals for 60 minutes and at 75- and 90-min post-exercise.

Change in GLUT temperature from pre-exercise was fitted to an exponential one phase decay model to determine logarithmic rate of heat dissipation and expressed as cooling half-life ($t_{1/2}$). $T_{1/2}$ was analyzed using repeated measures one-way ANOVA. All other data were analyzed using a repeated measures two-way ANOVA with the main effects of treatment, time, treatment x time, and period. Results were considered significant at $P < 0.05$ with trends discussed at $0.05 < P < 0.10$.

Figure 1. Heart rate pre- and post-exercise



Ambient temperature averaged 25.0 ± 4.7°C during the four-week study and ranged from 22.0°C to 28.5°C. Relative humidity averaged 60.3 ± 7.6% over the four-week study duration and ranged from 55.6% to 67.7%.

During the gallop portion of the exercise HR averaged 200±1 bpm. Recovery HR did not differ at any time point between treatment groups (figure 1). When pooled across timepoints, WALK HR were greater than all other treatments ($P < 0.05$), while STALL HR was greater than HOSE-10 ($P < 0.05$) but HOSE-0 was similar to both.

Treatments did not differ immediately post-exercise in RR (figure 2). At 5- and 10-min., HOSE-0 RR was less than WALK ($P < 0.05$). At 15 minutes, both HOSE-0 and HOSE-10 RR were less than WALK ($P < 0.05$). Treatments did not differ from 20 to 90 minutes in recovery. When RR were pooled across timepoints, WALK RR was greater than all other treatments ($P < 0.001$). STALL RR was greater than HOSE-10 and HOSE-0 ($P <0.001$). HOSE-10 and HOSE-0 RR were not different.

Figure 2. Respiratory rate pre- and post-exercise

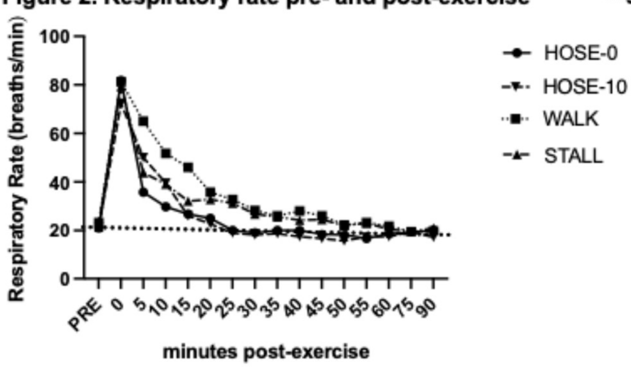


Figure 3. Gluteal muscle temperature pre- and post-exercise

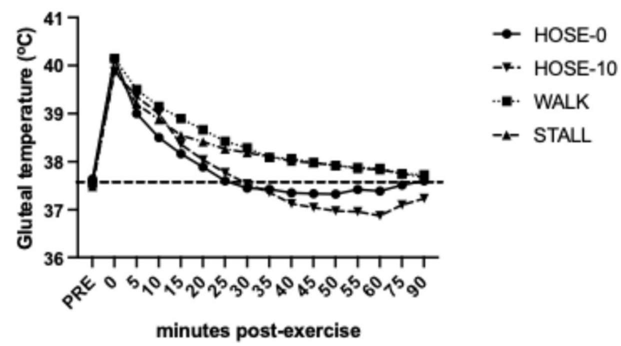


Figure 4. Gluteal muscle recovery post-exercise

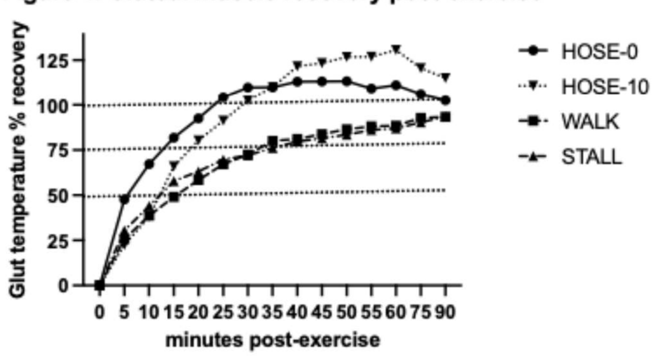
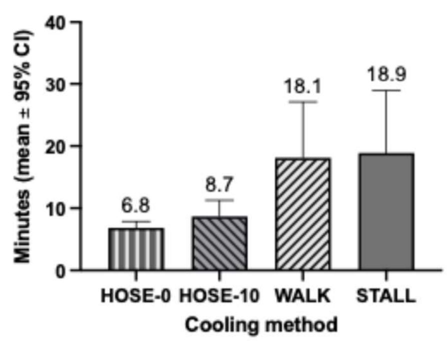


Figure 5. Gluteal muscle cooling rate half-life ($t_{1/2}$)



GLUT temperatures averaged 37.6 ± 0.2 °C (PRE) and 40.0 ± 0.2 °C at 0-min. post-exercise. GLUT temperature for HOSE-0 was less than WALK from five minutes until 50 minutes post-exercise ($P < 0.05$) and STALL from 20 to 50 minutes ($P < 0.05$) (figure 3). GLUT temperature for HOSE-10 was lower compared to WALK from 15 until 75 minutes ($P < 0.05$) and tended to be lower at 90 minutes ($P < 0.10$) and was lower than STALL from 30 minutes to 50 minutes ($P < 0.05$). Overall, STALL and WALK did not differ ($P > 0.10$) at any timepoint, nor did HOSE-0 and HOSE-10 ($P > 0.10$) except at 55 minutes ($P < 0.05$). HOSE-0 GLUT temperature returned to pre-exercise levels after 25-min and HOSE-10 after 30-min. WALK and STALL GLUT temperatures had not returned to pre-exercise levels after 90-min post-exercise (figures 3 and 4). Both HOSE-0 and HOSE-10 recovered to GLUT temps that were below pre-exercise levels. This was probably due to the wet horses standing under fans while stalled from 15-90 min. post-exercise. Hosing resulted in shorter cooling half-life (HOSE-0 $t_{1/2}$ 6.8 ±1.1 minutes, HOSE-10 $t_{1/2}$ 8.7 ±3.1 minutes) than either WALK ($t_{1/2}$ 18.1±10.8 minutes) or STALL ($t_{1/2}$ 18.9±12.0 minutes) ($p < 0.05$) (Figure 5). Although not statistically significant, there was a strong trend ($p = 0.062$) for HOSE-0 to have a shorter cooling half-life ($t_{1/2}$) than HOSE-10.

PTSM implanted in the middle gluteal muscle of 2-year-old thoroughbred racehorses produced no adverse reactions and were easy to use in a field setting to measure muscle temperatures post-exercise. Hosing made a large difference in the rate of cooling in these young racehorses after a routine training gallop resulting in a 2-3X faster cooling rate than hand-walking alone. Hosing immediately after exercise resulted in a slightly faster rate of cooling than walking 10 minutes before hosing, but the differences were small.

Kang H, *et al*. 2020. *Animals* **10** (12), 2274. doi:10.3390/ani10122274.
Kang H, *et al.* 2022. *Animals* **12** (10), 1267. doi: 10.3390/ani12101267

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MECHANICAL NOCICEPTIVE THRESHOLDS AND POSTURAL STABILITY IN HORSES FOLLOWING WHOLE-BODY VIBRATION THERAPY

Katherine L. Ellis, DVM, MS, DACVSMR, Camille Morris, Alison F. Harbold, Madeline E. Yokeley, Laura E. Franklin, Leslie Phelps, Katie Phelps, Valerie J. Moorman, DVM, MS, PhD, DACVS-LA

Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

Whole-body vibration (WBV) therapy involves standing on a vibrating platform and has been used in both human and equine athletes. WBV therapy has been used in the equine athlete during rehabilitation from injury and to help maintain core strength. Back pain in horses is due to primary back disease or secondary to hindlimb lameness. Treating primary back pain in horses can be challenging and often requires a multimodal approach. In humans, WBV has been demonstrated to reduce back pain through the stimulation of non-nociceptive sensory fibers. The multifidus muscle is the major stabilizing muscle of the spinal column and has been shown to atrophy in humans and horses with back pathology. A significant increase in the cross-sectional area of the multifidus muscle following 30 days of WBV therapy has been shown in the horse. A positive correlation has been seen between increased cross-sectional area (CSA) of the multifidus muscle and improved postural stability following rehabilitation exercises. The objectives of this study were to determine the effects of WBV on thoracolumbar pain, multifidus muscle CSA, and postural stability in horses with thoracolumbar pain.

Ten client-owned horses with thoracolumbar pain were enrolled in the study. Horses were excluded if they had their back treated with corticosteroids or biologics, mesotherapy, or shockwave therapy within the previous 3 months, if they had chiropractic work or acupuncture performed within the previous 30 days, or if they had received methocarbamol, gabapentin, or any non-steroidal anti-inflammatories within the previous 30 days. All horses underwent radiographs (Sound, Fusion Equine DR) of the thoracolumbar dorsal spinous processes and ultrasound (GE Logiq E, General Electric) of the thoracolumbar articular process joints (T18/L1 through L5/L6) as well as the multifidus and longissimus musculature. All imaging was performed and evaluated by a diplomate in the ACVSMR experienced in musculoskeletal imaging. Abnormalities (narrowing/impingement of the dorsal spinous processes, osteoarthritis within the thoracolumbar articular process joints, fibrosis/mineralization/muscle tearing) were graded subjectively using the following scale from 0 to 5: no pathology (0), mild (1), mild to moderate (2), moderate (3), moderate to marked (4), marked (5). Mechanical nociceptive thresholds (MNTs) were determined using a digital pressure algometer (FPX 100, Wagner Instruments) along the left and right sides of the back, approximately 2cm from midline at the thoracic spine (T13, T18), lumbar spine (L3, L6), and sacrum (S2). Measurements were taken in triplicate and averaged at each site. The same observer performed all MNT measurements. Mechanical nociceptive thresholds were performed prior to study initiation (day 0), 24 hours after first vibration therapy session (day 1), at day 14, and at day 30. Ultrasound evaluation of cross-sectional area (CSA) the multifidus muscle bilaterally at T12, T14, T16, T18, L2, and L4 was performed on days 0 and 30. Postural sway was measured using a previously validated portable media device (PMD). To collect postural sway data, each horse was fitted with a surcingle and the PMD (iPod touch 5th generation, Apple) was attached/stabilized to the surcingle with Velcro straps (Figure 1). Postural sway was collected under two circumstances: (1) normal square stance on flat ground and (2) square stance with all four feet on firm balance pads (SureFoot, green pads). Five 10-15 second trials were collected for each condition. Medio-lateral (ML) and cranio-caudal (CrCd) accelerations were collected at 100 Hz using a commercially available data logger application, and range of motion (ROM) was determined for each trial. Postural sway data was collected prior to study initiation (day 0) and at 30 days. All horses stood on a mobile WBV plate (EquiVibe) for 30 minutes once daily, 5 days per week for 4 weeks for a total of 20 treatments. A frequency of 40Hz, amplitude of 0.8mm, and an acceleration of 4.9m/s^2 using a vertical-type vibration was used for all sessions for all horses. Statistical analysis was performed using commercial software (SAS 9.4 and STATA 13.1). A linear mixed model was used to assess MNTs with fixed factors of day, location, and a day by location interaction effect, and a covariate for baseline MNT and random intercepts for horse and side within horse. To test for association of imaging scores with MNT, an imaging score covariate with all two- and one three-way interaction effects were added to the model. Multiple comparisons were performed using Tukey's test. Satterthwaite degrees of freedom method and REML estimation were used. Kruskal-Wallis tests were used to compare symmetry values between days (averaged over sites) and for each site separately. For multifidus muscle CSA analysis, a linear mixed model analysis was performed with time, side (left/right), and spinal location as fixed factors and horse as a random factor. For postural sway analyses, linear mixed model analysis was performed with day and condition (square stance/pads) as fixed variables and horse as a random variable. A significance of $P < 0.05$ was used for all analysis.

There was no significant association of overall (P=0.639) or cumulative image scores with MNT scores at any location or day. There was a significant effect of day on MNT. MNTs across all locations were significantly lower compared to baseline on day 1 (p<0.001) and day 14 (p=0.004). MNTs were significantly higher than baseline on day 30 (p<0.001). Across all spinal locations, there was a significant increase in multifidus muscle CSA on day 30 compared to day 0 (P = 0.0005). A significant decrease in mediolateral range of motion from day 0 to day 30 was present both during square stance (P <0.001). and when standing on pads (P <0.001) . There was no significant difference in craniocaudal, yaw, pitch, or roll range of motion from day 0 to day 30 either during square stance or when standing on pads.

The results of this study support that short-term (less than 14 days) use of WBV at 30 minutes once daily, 5 days per week in horses with thoracolumbar pain may result in increased pain. Long-term use (4 weeks) is needed to reduce thoracolumbar pain in horses. Four weeks of once daily WBV is sufficient to improve postural stability and increase multifidus muscle CSA.

Figure 1. Photograph of study horse standing on balance pads with PMD secured to surcingle for collection of postural sway data

Figure 2. Mean MNTs averaged across all sites by day. Mean MNT denoted by black circles. Standard deviation indicated by black bars. Asterisks indicate a significant difference compared to Day 0

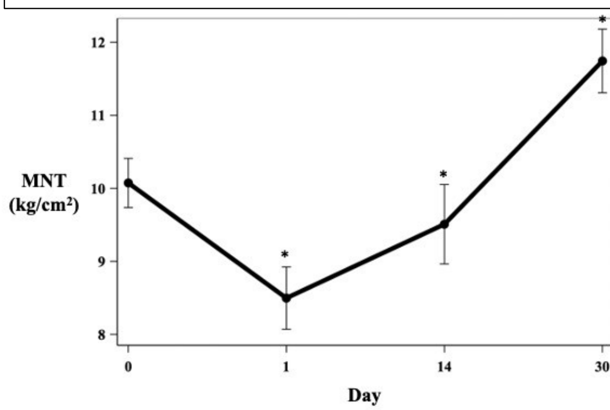

Table 1. Postural sway data comparing day 0 and day 30 of WBV during flat square stance and standing on pads.

		Flat Square Stance			Balance Pads		
		Day 0	Day 30	P-value	Day 0	Day 30	P-value
ML ROM (m/s^2)	mean	0.35	0.3	<0.001	0.61	0.38	<0.001
	95% CI	0.31 - 0.39	0.27 - 0.33		0.49 - 0.76	0.34 - 0.43	
CrCd ROM (m/s^2)	mean	0.25	0.24	0.224	0.41	0.32	0.224
	95% CI	0.23 - 0.28	0.21 - 0.26		0.34 - 0.51	0.29 - 0.37	
Yaw ROM (°)	mean	2.01	1.69	0.086	3.61	2.81	0.086
	95% CI	1.61 - 2.52	1.37 - 2.10		3.06 - 4.26	2.44 - 3.23	
	mean	0.82	0.79	0.332	1.30	1.01	0.332

Pitch ROM (º)	95% CI	0.68 - 0.99	0.67 - 0.93		1.07 - 1.58	0.85 - 1.19	

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CONSISTENCY OF CIRCULATING MYOSTATIN IN THOROUGHBREDS GENOTYPED FOR A SINE INSERTION IN THE MYOSTATIN GENE PROMOTER

Katherine Hanousek, Victoria O'Hara, Dominique Riddell, Richard J. Piercy.

Comparative Neuromuscular Diseases Laboratory, Royal Veterinary College, London, UK

The secreted growth differentiation factor, myostatin, is a regulator of skeletal muscle growth that is negatively associated with bone density in humans and mice. Across various species, null mutations of the myostatin gene (*MSTN*) are associated with skeletal muscle bulk. In horses, a highly prevalent SINE mutation in the *MSTN* promoter affects circulating myostatin concentration, and is associated with optimal race distances and skeletal muscle fibre type distributions. Our prior data reveals that within genotyped horse groups, there is substantial variation in serum myostatin concentration, particularly in *MSTN* mutant heterozygotes and wild type horses. However, it is unclear whether an individual animal's serum myostatin concentration remains consistent or varies and whether myostatin expression might change throughout the year. If an individual horse's circulating myostatin concentration remains consistent there might be other genetic or environmental factors that influence its expression which might then be similarly associated with performance-related traits. Further, given its link to fracture risk in horses, monitoring of circulating myostatin concentrations might be valuable both in performance-optimisation and safeguarding of equine athletes. The purpose of this study was therefore to investigate the hypothesis that in Thoroughbred racehorses of defined *MSTN* genotype, myostatin expression remains consistent, with low intra-horse variation across multiple samples at different times of the year.

Residual EDTA blood samples were obtained from various animals within a group of 49 Thoroughbred racehorses under identical management at approximately monthly intervals between April 2023 and April 2024. Samples were obtained in the afternoon (3-3:30pm) following light exercise in the morning (7-8:30am). Plasma was separated and frozen at −80 °C within 48 hours of sampling. Signalment data were recorded. DNA was extracted from the cellular component and genotyped for the SINE insertion in the *MSTN* promoter by PCR. Horses were classified as wild type, heterozygous, or homozygous for the *MSTN* SINE mutation. Plasma myostatin concentrations were measured (in duplicate) using a previously validated commercial ELISA (GDF-8/Myostatin ELISA: R&D systems), with serial dilutions confirming linearity and with three sentinel samples (one per genotype) repeated across plates.

Data analyses were conducted in R (R Core Team, 2022, Vienna, Austria), with linear mixed-effects models used to assess the effect of genotype, season, sex, age, and their interactions on plasma myostatin concentrations; horse and ELISA plate were included as random effects. Normality was checked using histograms of the residuals, and significance was assessed via type III ANOVA. The coefficient of variation (CV%) of plasma myostatin concentration was calculated for each individual for which 3 or more plasma samples were available. Further, in order to examine the possible influence of day to day haemodynamic changes on plasma myostatin concentration, we additionally measured plasma albumin in the three horses per genotype with the highest CV% to calculate a myostatin: albumin ratio, and compared this with the un-normalised myostatin CV%. We also examined the effect of handling and storage.

The handling and storage conditions used in the study had no effect on plasma myostatin concentration. Of the 49 horses there were 27 geldings, 16 mares and 6 stallions, with a median (IQR) age of 3.5 (1.5) years at time of initial sampling. The mean and standard deviation of myostatin concentration was 459.2 pg/ml (111.4) for heterozygous horses (n = 23), 100.5 pg/ml (33.3) for homozygous horses (n = 8) and 725.7 pg/ml (220.4) for wild type horses (n = 18). As expected, genotype had a highly significant effect on plasma myostatin concentration ($p < 0.001$ between all genotypes). There was no significant effect of season sampled ($p = 0.079$), and no significant interaction between season sampled and genotype ($p = 0.36$). Date sampled, horse sex and age had no significant effect on myostatin concentration with no significant interaction with genotype. The mean CV% for an individual horse of each genotype was 17% (SD = 6%) for heterozygous horses, 18% (SD = 9%) for wild-type horses and 23% (SD = 10%) for homozygous horses. There was no significant difference in CV% between the genotypes ($p > 0.05$). Normalising the plasma myostatin concentration to albumin did not change the CV% for the sub-population of horses (mean CV% for myostatin concentration: 25% (SD = 6%) vs albumin-corrected values: 26% (SD = 6%; $p = 0.36$). Over the one-year sampling period, circulating myostatin concentrations remained stable within individual horses, with no significant effects of date, season, sex, or age on myostatin levels ($p > 0.05$).

Whilst a limitation of this study is the lack of recent exercise, fitness or training history of the horses sampled, all horses were within a narrow age range (2 to 7 years), and were all in race training with similar management. Our data therefore likely represents the racing population. We reveal that whilst there is some variation within an individual horse, there is consistency: i.e. some horses have relatively lower or higher plasma myostatin than other horses, within their defined *MSTN* genotype.

The reason for the relative consistency (and variation) in plasma myostatin within horses remains unknown but might be attributed to other genetic or environmental factors. Given the strong association between *MSTN* mutations and race distance aptitude in Thoroughbreds, it will be of interest to examine the effect of circulating myostatin concentration within genotyped groups, on various performance related traits such as optimal race distance, muscle size and fibre type differences: its measurement might help refine associations with genotype alone. Additionally, with the potential role of myostatin in bone health and fracture risk, as observed in other species, understanding its variability could have significant implications for equine welfare: circulating myostatin levels would be an easily measured marker if its association with fracture risk was defined.

In summary, our study highlights the relative consistency of circulating myostatin concentrations within individual Thoroughbreds over time, and confirms the major differences between *MSTN* genotyped groups. Further work is required to determine whether circulating myostatin concentration is associated with performance and fracture risk in horses.

Additional Notes

Forename	***Surname***	***Email***
Francisco	Alejandro Uzal	fauzal@UCDAVIS.EDU
Warwick	Bayly	wmb@wsu.edu
Maëlle	Bonhomme	Maelle.Bonhomme@uliege.be
Lise	Charlotte Berg	lcb@sund.ku.dk
Cindy	Cole	Cynthia.Cole@uky.edu
Sandro	Colla	Sandro.Colla@colostate.edu
Erin	Contino	erinkcontino@gmail.com
Michael	Davis	michael.davis@okstate.edu
Guillaume	Dubois	guillaume@arioneo.com
Mary	Durando	mdurando2004@yahoo.com
Matt	Durham	matt@platinumperformance.com
Katie	Ellis	Katie.Ellis@uga.edu
Jonathan	Foreman	jhf@illinois.edu
Sam	Franklin	sam.franklin@adelaide.edu.au
Katherine	Hanousek	khanousek5@rvc.ac.uk
Sherry	Johnson	sherryjdvm@gmail.com
Chris	Kawcak	Christopher.Kawcak@ColoState.EDU
Rob	Keene	rob.keene@boehringer-ingelheim.com
Melissa	King	Melissa.King@colostate.edu
David	Lambert	stridesafeusa@gmail.com
Renaud	Leguillette	rleguill@ucalgary.ca
Erica	McKenzie	Erica.McKenzie@oregonstate.edu
Jessica	Morgan	jmmorgan@ucdavis.edu
Ashleigh	Morrice-West	ashleigh.morrice@unimelb.edu.au
Carolien	Munsters	carolien@munsters.nl
Cris	Navas	navasdes@vet.upenn.edu
Laura	Nath	lauracnath@gmail.com
Crevier-Denoix	Nathalie	nathalie.crevier-denoix@vet-alfort.fr
Joe	Pagan	pagan@ker.com
Allen	Page	a.page@uky.edu
Thilo	Pfau	thilo.pfau@ucalgary.ca
Gene	Pranzo	gmpranzo@genepranzo.com
Sarah	Reuss	sarah.reuss@boehringer-ingelheim.com
Stephen	Smith	kitmanlabs.com
Susan	Stover	smstover@ucdavis.edu
Emmanuelle	Van Erck	evanerck@esmp.be
Gunther	van Loon	Gunther.VanLoon@UGent.be
Lidwien	Verdegaal	lidwien.verdegaal@adelaide.edu.au
Fe ter	Woord	fterwoort@esmp.be
Dominique	Votion	Dominique.Votion@uliege.be

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