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International Conference on Equine Exercise Physiology 2014

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Scientific Abstracts
International Conference on Equine Exercise Physiology 2014

Foreword and Acknowledgements

This publication is a compilation of all Research Abstracts presented at the Ninth International Conference on Equine Exercise Physiology. Unlike previous ICEEP conferences there will not be a conference proceedings of full length manuscripts. These abstracts succinctly summarise a wide array of investigations relevant to the equine athlete, and will be useful to veterinarians and others involved in management of horses used for sport, work and competition. The abstracts encompass the topics of Applied Physiology; Biochemistry, Haematology, Endocrinology, and Thermoregulation; Cardiovascular and Respiratory; Muscle and Bone; Nutrition; Genomics, Proteomics, and Metabolomics; Biomechanics and Locomotion; as well as Physiotherapy, Rehabilitation, and Equitation science.

The International Committee of ICEEP publishes these abstracts so that the most recent scientific information is available to a wide audience, including veterinarians, physiotherapists, trainers, owners and riders.

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APPLIED PHYSIOLOGY: NEW TECHNOLOGY IN EQUINE EXERCISE PHYSIOLOGY

EVALUATION OF MASKS FOR FIELD TESTING OF EXERCISING HORSES

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Introduction: The ability to accurately assess equine oxygen consumption (VO_2) under field conditions has been limited by the need for unrestricted gas exchange.

Methods: Two variations of a mask and an associated electronics control module (ECM) were designed to enable breath-by-breath measurement of airflows with two 8.0 cm diameter pneumotachometers located 7.5 cm in front of each narus and connected to differential pressure transducers mounted on the outside of the mask. The ECM was comprised of electronics for signal filtering to the flow transducers, an oxygen sensing cell, and an analog-to-digital converter all powered by a lithium-ion battery. The battery also powered a pump connected to gas sampling ports between the nares and pneumotachometers. Airflow and oxygen content of inspired and expired gases were recorded through the ECM and electronically transferred to a notebook. VO_2 was determined from these recordings by an operator using a customized software analysis program. One mask encased the lower head (E). The other left the jaw free so horse could wear a bit and be ridden (R). Multiple treadmill exercise tests were undertaken by 6 horses to measure VO_{2max} and blood gases. Each mask was worn twice and results compared to those from an open flow-through system (O) by 2-way RMANOVA ($P<0.05$). Utility of the system was evaluated using the intraclass correlation coefficient of 4 independent raters.

Results: Blood gases and VO_{2max} (152.0 ± 4.0 (mean ± SEM; O), 147.7 ± 4.3 (E), 150.7 ± 3.3 (R) ml/(kg.min) were not different between masks. VO_2 measures were reproducible for each mask. Agreement between the 4 raters was excellent (intraclass correlation coefficient = 0.99).

Conclusions: Masks capable of measuring VO_2 during field exercise were developed, tested and found accurate by multiple users.

Ethical Animal Research: Studies performed were approved by the Institution's Animal Care and Use Committee (protocol #3807). **Sources of funding:** Institutional sources. **Competing interests:** Washington State University has filed notice of its intent to apply for a patent for facemask used in this study.

ALTERED MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION CAPACITY IN HORSES SUFFERING FROM POLYSACCHARIDE STORAGE MYOPATHY

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Introduction: Exertional myopathies are a common cause of exercise intolerance in the equine athlete, and Polysaccharide Storage Myopathy (PSSM) is a widely described muscular disease. It is characterized by an accumulation of abnormal glycogen in myofibers due to a genetic defect in the skeletal muscle glycogen synthase (GYS1) enzyme. We hypothesized that energy production through oxidative phosphorylation (OXPHOS) in muscular mitochondria might be impaired in type-1 PSSM-affected horses.

Methods: Eight horses with a history of exertional rhabdomyolysis were tested for the GYS1 mutation. Muscle biopsies were collected and used for histological analysis and high resolution respirometry (HRR). HRR values from 3 groups of horses (5 PSSM-positive horses, 3 horses with a history of myopathy but PSSM-negative and 16 healthy controls) were compared using a linear mixed model to take into account repeated (2–3 times) measurements made for each horse.

Results: In 5/8 horses histology revealed an accumulation of abnormal glycogen in myofibers. These 5 horses also tested positive for the GYS1 mutation. A severe depression of maximal OXPHOS capacity was observed by HRR in 7/8 horses with exertional rhabdomyolysis, with lower values in PSSM-positive cases (4/5).

Conclusions: Our study shows a severely decreased OXPHOS capacity in PSSM-affected horses. PSSM is considered primarily a defect in glycogen synthesis but altered OXPHOS might play a central role in its pathogenesis.

Ethical Animal Research: All study procedures were approved by the Animal Ethic Commission of the University of Liege. **Sources of funding:** University of Liège. **Competing interests:** none.

APPLIED PHYSIOLOGY: DRUGS IN EQUESTRIAN SPORT AND EXERCISE

MODIFICATION OF THE METABOLOMIC PROFILE INDUCED BY CHRONIC GROWTH HORMONE ADMINISTRATION IN A DOPING MODEL

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Introduction: Horses performance can be enhanced via growth hormone (GH) doping. The detection time for GH in blood is too short to allow detection during competition. GH not only affects the proteosynthesis in cells and tissues but can also cause long term metabolic variations. This study aimed to show the metabolomic modifications occurring after three months of GH treatment.

Methods: The study was performed on 20 Welsh ponies. 10 were injected with REPORCIN TM, an approved recombinant GH (30 $mg.kg^{-1}$ per day) for 3 months and the other 10 comprised the control group. Blood samples were collected before GH treatment (T0), then 3 months later (T3). The NMR spectra acquired on the 40 serum samples were classified according to the factors (season, GH treatment) using the supervised multivariate orthogonal projection on latent structure (OPLS) method. A first OPLS model was computed with the control ponies (T0-T3). The predictive component of this model was subtracted from the whole data (treated, controls at T0 and T3) to only retain the GH-dependent variations.

Results: Before filtering seasonal effects, glucose, lipids and phospholipids were metabolites discriminating between T0 and T3 samples. After removing the seasonal effect, the OPLS model revealed the effect of GH treatment on protein metabolism through the modification of aromatic amino acids and branched chain amino acids. The variations of other metabolites (glycoproteins and lactate) were enhanced.

Conclusions: This study showed that chronic GH injection induced systemic metabolism modifications that can be detected using the NMR metabolomic method without neglecting the misleading effect of season.

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Ethical Animal Research: Studies approved by "Comité regional d'éthique pour l'expérimentation animale Centre Limousin" Saisine N° CL2008_015. **Sources of funding:** French Agency against Doping (AFLD). **Competing interests:** none.

PRE-RACE ADMINISTRATION OF FUROSEMIDE INDUCES A LINEAR DOSE-DEPENDENT INCREASE IN PLASMA TOTAL CO_2 CONCENTRATION IN THOROUGHBRED RACEHORSES

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Introduction: Furosemide is widely administered in North America for the prophylactic treatment of exercise-induced pulmonary hemorrhage. Plasma total CO_2 concentration ($ctCO_2$) provides a clinically useful screening test for the presence of metabolic acid-base disturbances, including the pre-race administration of alkalinizing agents to racehorses. Some racing jurisdictions account for the metabolic alkalosis and increased plasma $ctCO_2$ induced by furosemide administration. The purpose of this study was to determine the relationship between pre-race furosemide dose and pre-race plasma $ctCO_2$ in Thoroughbred racehorses.

Methods: Jugular venous blood was collected anaerobically into 3 mL partially evacuated plastic tubes containing lithium heparin from 226 Thoroughbred horses administered a variable dose of furosemide (150–500 mg IV, n = 217) 3–4 hours previously or not administered furosemide (n = 9).

Plasma $ctCO_2$ was calculated from the measured values for pH and pCO_2 using a blood gas and pH analyzer [Radiometer ABL 700] and the Henderson-Hasselbalch equation. Data was analyzed using multivariable linear regression and $P<0.05$ was significant.

Plasma $ctCO_2$ in mmol/L increased linearly ($P = 0.0006$) with furosemide dose in milligrams adjusted for sex (female, stallion, gelding), such that $ctCO_2 = 33.38 - 0.71(\text{if female}) - 0.63(\text{if stallion}) + 0.0040 \times (\text{furosemide dose})$.

Conclusions: Intravenous administration of furosemide (150–500 mg) produces a linear dose-dependent increase in $ctCO_2$ in plasma. High intravenous doses of furosemide (500 mg) have the potential to increase plasma $ctCO_2$ to near the threshold value used to identify the pre-race administration of alkalinizing agents. This finding supports an allowance of 2.0 mmol/L for furosemide administration used by some racing jurisdictions in North America.

Ethical Animal Research: Samples and data were collected during race day procedures currently in place. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Indiana Horse Racing Commission. **Competing interests:** none.

EFFICACY OF INTRAVENOUS DEXAMETHASONE VERSUS PLACEBO IN EXPERIMENTAL FOOT LAMENESS IN HORSES

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Introduction: There are no refereed blinded controlled documentations of the skeletal analgesic efficacy of intravenous dexamethasone. The objective was to test the hypothesis that intravenous dexamethasone is more efficacious in alleviating lameness than placebo in a reversible adjustable heart bar shoe model of equine foot pain.

Methods: Eight healthy horses (7 Thoroughbreds, 1 Warmblood, aged 6.5 ± 3.3 years, age range 2–11 years) underwent weekly intravenous treatments 1 hour after lameness induction. Treatments were isotonic saline placebo at 1 ml/45 kg bwt, or dexamethasone (2 mg/ml in polyethylene glycol) at 0.045 mg/kg bwt, a typical dose used twice daily in the USA for anti-inflammatory effect. Treatments were randomly assigned and administered by a co-investigator who had no input on Heart Rate (HR) and Lameness Score (LS) measurements. Another investigator unaware of treatment assignments monitored HR and LS every 20 min for 5 hours after lameness induction and then hourly through 12 hours post-treatment. One week later treatment assignments were reversed and the experiment was repeated. Repeated measures ANOVA and post hoc Tukey's test were used to identify significant effects at $P<0.05$.

Results: After treatment, mean HR and LS were not different between treatments ($P>0.05$). HR and LS remained elevated over pre-lameness values throughout the 13-hour post-lameness monitoring period ($P<0.05$).

Conclusions: It was concluded that intravenous dexamethasone was no more effective than saline placebo. Neither treatment was effective at alleviating lameness over a 12-hour post-treatment period in this model. Dexamethasone at 0.045 mg/kg bwt should not affect acute foot lameness during equine exercise or performance.

Ethical Animal Research: The study was approved by the University of Illinois Institutional Animal Care and Use Committee. **Sources of funding:** Fédération Equestre Internationale. **Competing interests:** none.

EFFICACY OF PHENYLBUTAZONE VERSUS FIROCOXIB IN EXPERIMENTAL LAMENESS IN HORSES

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Introduction: There are few refereed blinded controlled documentations of skeletal analgesic efficacy of firocoxib, a selective COX-2 inhibitor. The objective was to test the hypotheses that intravenous firocoxib and phenylbutazone are comparable in efficacy and that both are more efficacious in alleviating lameness than placebo in an adjustable heart bar shoe model of equine foot pain.

Methods: Eight healthy adult horses (5 Thoroughbreds, 3 Quarter horses, mean age 8.3 ± 0.9 years, age range 4–13 years) underwent weekly intravenous treatments 1 hour after lameness induction. Treatments were isotonic saline placebo (1 ml/45 kg body weight), phenylbutazone (4.4 mg/kg), and firocoxib (label dosage 0.09 mg/kg). Treatments were randomly assigned and administered by co-investigators who had no input on Heart Rate (HR) and Lameness Score (LS) measurements. Another investigator who was unaware of treatment assignments monitored HR and LS every 20 min for 5 hours after lameness induction and then hourly through 12 hours after treatment. One and two weeks later treatments were shuffled and the experiment was repeated. Repeated measures ANOVA and post hoc Tukey's test were used to identify analgesic effects at a significance level of $P<0.05$.

Results: HR and LS were not different between saline and firocoxib. Post-treatment phenylbutazone HR (2.3–3.3, 5, and 10 hours) and LS (2.3–8 hours) were lower than saline ($P<0.05$). Phenylbutazone HR (10 and 12 hours) and LS (1.67–11 hours) were lower than firocoxib ($P<0.05$).

Conclusions: It was concluded that firocoxib at this label dosage was not different than placebo and that phenylbutazone was more effective than firocoxib and saline.

Ethical Animal Research: Study approved by the University of Illinois Institutional Animal Care and Use Committee. **Sources of funding:** Fédération Equestre Internationale. **Competing interests:** none.

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DOES PHENYLBUTAZONE ADMINISTRATION CONTRIBUTE TO GASTRIC PERMEABILITY AND ULCERATION IN HORSES?

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Introduction: Gastric ulcers are commonly found in equine athletes and there is evidence suggesting phenylbutazone may increase the incidence. In human athletes, gastric permeability has been shown to increase with non-steroidal anti-inflammatory use with potential to negatively affect performance and recovery. The aim of this study was to examine the effects of phenylbutazone on gastric mucosa and permeability.

Methods: 18 Arabian horses were examined via gastroscopy on day 0, 42, 49, 56, and 63, with ulceration in both the squamous and glandular regions scored from 0 to 4. For seven days commencing after the day 42 evaluation, horses received 4.4 mg/kg BW phenylbutazone twice daily PO. On day 49, sucrose (1 g/kg bwt) was administered via nasogastric tube, with blood and urine samples collected for 90 min and 4 h, respectively, to assess gastric permeability. Data were analyzed using SAS 9.2 with Proc MIXED.

Results: Ulcer scores increased from day 42 to day 49 ($P<0.0001$) with prevalence of grade 2 or greater ulceration being 33% on day 42 compared with 94% on day 49. Scores decreased from day 49 to 56 and, though continuing to decrease, had not returned to baseline scores by day 63. Squamous scores increased from day 0 to day 49 and remained elevated through day 63, while glandular scores were highest on day 49 but had decreased by day 56 ($P<0.05$). Sucrose concentration increased ($P<0.0001$) in both plasma and urine after phenylbutazone administration.

Conclusions: Besides exacerbating gastric ulcers, phenylbutazone contributes to gastric permeability and, particularly when combined with strenuous exercise, may impair recovery and increase illness susceptibility.

Ethical Animal Research: All methods were approved by the Michigan State University Institutional Animal Care and Use Committee (approval number 10/11-216-00). **Sources of funding:** Zinpro Corporation. **Competing interests:** none.

FUROSEMIDE ADMINISTRATION AFFECTS MINERAL EXCRETION IN EXERCISED THOROUGHBREDS

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Introduction: Furosemide is widely administered to Thoroughbred and Standardbred racehorses to reduce the incidence and severity of EIPH. The magnitude and duration of mineral loss following furosemide administration has not previously been measured in horses.

Methods: Six fit Thoroughbreds (age 6.8 ± 1.2 y; bwt 585 ± 19 kg) were used in a 3 x 3 Latin square design trial to measure the effect of furosemide on urinary and fecal mineral excretion for 24 h before (–24 h) and 24 h, 48 h and 72 h after a standardized exercise test (SET) on an inclined (3°) high speed treadmill. The treatment groups consisted of an untreated control (C) and two groups (F and FE) which received furosemide (0.5 mg/kg, IV) 4 h before the SET. C and F were fed 60 g/d NaCl and (FE) was fed 200 g/d of an electrolyte mix (14 g K, 37.7 g Na and 43.3 g Cl) for 21 days before the SET. Following the SET the FE group was supplemented with an additional 10 g/d Ca and 10 g/d Mg.

Results: Urinary Ca, P, Na and Cl excretion increased 24 h post treatment in the F and FE groups ($P<0.05$). 72 h Ca and Cl balance was decreased in F ($P<0.05$) and 72 h Mg and Cl balance was decreased in FE ($P<0.05$). The digestibility of P and Mg was decreased in FE ($P<0.05$) and Na digestibility was increased in F and FE ($P<0.05$).

Conclusions: A single dose of furosemide negatively affected calcium balance in horses for 72 h post administration. Further research is needed to assess the effect of chronic furosemide use in racehorses on mineral balance and soundness.

Ethical Animal Research: Study approved by the Kentucky Equine Research Institutional Animal Care and Use Committee. **Sources of funding:** Kentucky Equine Research. **Competing interests:** none.

EFFECTS OF PRELOADING WITH NaCl, KCl, $NaHCO_3$, OR $KHCO_3$ ON PLASMA ELECTROLYTE CONCENTRATIONS AND RUN TIME DURING A HIGH INTENSITY EXERCISE TEST: A PILOT STUDY

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Introduction: $NaHCO_3$ has been administered prior to racing in an attempt to enhance performance but it remains unclear whether potential ergogenic effects may be due to increased buffering capacity or expanded extracellular fluid volume.

Methods: Solutions of 1 g/kg of NaCl, KCl, $NaHCO_3$, or $KHCO_3$ in 8 L of water were administered via a nasogastric tube 60 min prior to a high intensity treadmill exercise test (12 m/s to fatigue) to 4 horses in a randomized fashion. Changes in mixed venous plasma electrolyte concentrations (NOVA Biomedical analyser) and run time to fatigue were compared by repeated measures ANOVA.

Results: Administration of Na and K salts increased Na and K concentrations before and during maximal exercise, respectively. Administration of bicarbonate salts attenuated acidosis and the decrease in bicarbonate concentration during exercise, as compared to Cl salts. Of interest, run time to fatigue was longer ($P<0.001$, power = 0.98) after administration of KCl (5.1 ± 0.6 min) as compared to both bicarbonate salts (3.7 ± 0.6 and 3.6 ± 0.8 min for $NaHCO_3$ and $KHCO_3$, respectively), but was not different than run time after NaCl administration (4.3 ± 0.8 min).

Conclusions: Preloading with large amounts of Na and K salts produced expected changes in mixed venous Na and K concentrations but preloading with bicarbonate did not improve performance. Observation of longer run times to fatigue after administration of NaCl or KCl was unexpected and warrants further investigation.

Ethical Animal Research: Animal use approved by the Animal Use Committee of Michigan State University. **Sources of funding:** Department of Large Animal Clinical Sciences, Michigan State University. **Competing interests:** none.

APPLIED PHYSIOLOGY: TRAINING

EFFECT OF ACUTE EXERCISE AND AEROBIC CONDITIONING ON THE FAECAL PH OF HORSES

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Introduction: Repeated monitoring of faecal pH may be useful in detecting the effects of exercise and conditioning on the fermentative activity within the large intestine. This study investigated the effect of a

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maximal incremental exercise test (MIET) and aerobic conditioning on the faecal pH of untrained horses.

Methods: Fourteen equines were randomly divided into two experimental groups. The control group consisted of 7 horses that remained untrained. The second group underwent physical training (n = 7). The trained equines underwent two MIETs, once before and once after exercise conditioning. The conditioning period lasted 6 weeks and consisted of 3 weekly sessions of exercising on a treadmill at 70% of lactate threshold. All horses were fed the same diet. To evaluate the effect of exercise on faecal pH, stool samples were collected before and 48 h after the MIETs (before and after training) for determination of fecal pH. To determine chronic effects, weekly stool sampling was performed at 0 (immediately before), 7, 14, 21, 28, 35 and 42 days of training. One-way repeated measures ANOVA followed by Dunnett's test, the paired t-test (MIET) and the unpaired t-test were performed for comparisons between the groups at the different times.

Results: Faecal pH decreased from day 28 in the trained horse group compared to controls. pH values were 7.57 ± 0.10 and 7.27 ± 0.24 (before) and 7.97 ± 0.14 and 6.75 ± 0.15 (after) for the control and trained groups respectively. There was no change in faecal pH after the MIET.

Conclusions: It appears that physical conditioning reduced faecal pH of horses.

Ethical Animal Research: The Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation was approved by the institutional animal care and use committee of the university (CEUA, Process: 016842/13). **Sources of funding:** FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo (Process 2013/14703-3). **Competing interests:** none.

PRELIMINARY ASSESSMENT OF DRESSAGE ASYMMETRY WITHIN SITTING TROT AND SHOULDER-IN

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Introduction: Despite dressage popularity, little research exists in relation to the rider's posture and effect on aids given to the horse. The aim was to investigate asymmetry during sitting trot and shoulder-in between varying rider abilities.

Methods: Full body kinematics were collected at 120 Hz (XSENS MVN Biomech) for 10 novice (age 28 ± 12 years, height = 168 ± 7 cm) and 10 advanced (age 29 ± 6 years, height = 163 ± 4 cm) predominantly right handed participants, with one left handed rider in each group. Range of motion for right hip, left hip, right knee, left knee, right ankle, left ankle, ASIS vertical movement, trunk rotation were generated in Visual 3D.

Results: Independent t-tests demonstrated significant differences between groups within right and left hip external rotation when straight ($P < 0.001$, $d = 2.52$; $P = 0.02$, $d = 1.25$), shoulder-in right ($P = 0.01$, $d = 1.47$; $P = 0.02$, $d = 1.31$), only significant in right hip within shoulder-in left ($P = 0.01$, $d = 1.30$). Axial rotation to the right was found in all conditions, with the range significantly ($P<0.05$) increased between groups in straight and shoulder-in right, as did right ASIS vertical excursion when riding straight ($P = 0.01$, $d = 1.90$), and left ASIS excursion within shoulder-in left ($P = 0.04$, $d = 1.03$).

Conclusions: Right trunk axial rotation is considered closely linked to right hand dominance, rotating the rider's trunk to the dominant rein aid. Left shoulder-in scored higher which may be because right trunk rotation is one of the aids given to the horse when executing this dressage movement.

Ethical Animal Research: Study protocols approved by the University of Central Lancashire, before commencing the study. **Sources of funding:** none. **Competing interests:** none.

EFFECT ON VLa4 OF REDUCED EXERCISE PERIODS DURING CONDITIONING OF HORSES

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Introduction: One week of reduced workload between 3 periods of 2 weeks of conditioning (CP) 5 times/fortnight at VLa10 for two bouts of 5 minutes allowed for a continuous increase of VLa4, but the extent was smaller than in another study with the same conditioning program but for the recovery week (VLa4, VLa10: horse's speed, which produced a blood lactate concentration of 4 and 10 mmol/l).

Methods: Six Purebred Arabians were submitted to the same conditioning protocol of the studies mentioned above except for increasing the duration of treadmill exercise in each CP by 2.5 minutes (2 x 10 minutes in the third CP). A standardized exercise test (SET) was performed at the beginning of CP to determine VLa4 and VLa10, and repeated after the week with reduced workload after each CP. Exercise speed was individually adapted to the new VLa10 for every CP. The effects of conditioning on VLa4 were examined using repeated measures analysis of variance. Significant results were analyzed further with Fisher's test.

Results: Mean VLa4 increased after the first CP ($P<0.001$), fell between first and second CP ($P<0.001$) and remained at this level after the third CP ($P>0.05$).

Conclusions: This conditioning program did not allow for VLa4 increases of the magnitude observed in other studies with the same exercise protocols but without the week with reduced workload and increases in exercise duration. It is possible that the decline of VLa4 of the horses after the first CP was due to overtraining (simultaneous increase of speed and duration of exercise). The effect of recovery from training needs further studies.

Ethical Animal Research: All procedures were approved by the Bioethical Committee of the University of La Plata, Argentina. **Sources of funding:** Verein zur Förderung der Forschung im Pferdesport, Germany. **Competing interests:** none.

DOES SWIM TRAINING IMPROVE ATHLETIC PERFORMANCE OF MANGALARGA MARCHADOR HORSES?

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Introduction: Swimming can be used for conditioning and rehabilitation of horses. Its main advantage is minimal musculoskeletal stress. The purpose of this study was to evaluate the influence of swimming on athletic performance of Mangalarga Marchador horses.

Methods: Eight horses (6.7 ± 2.9 years old, 426 ± 32 kg) conditioned to swim training were evaluated twice, before (M1) and after (M2) four weeks of training consisting of 60 minutes of hand-walking twice a week, alternating with marcha exercise with a rider for 40 minutes three days a week, and 20 minutes of marcha with a rider each day of the weekend. Four animals were randomly selected after M1 as a test group and were submitted to swim training for 15 minutes twice a week in a lake. During M1 and M2, heart rate, hematocrit, AST, CK and lactate were recorded before (T0) and after 5 (T1), 30 (T2) and 120 (T3) minutes of exercise (marcha gait for 40 minutes). Variables were analyzed for normality with Kolmogorov-Smirnov test. Comparisons between M1 and M2 and between control and test groups on each moment were made using paired t-test ($P<0.05$).

Results: No differences were observed for HR, hematocrit, lactate and AST. Serum CK was significantly different for the test group between

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M1 and M2 for T1 (P = 0.0175), T2 (P = 0.0498) and T3 (P = 0.0226) with higher values for M2; also significant differences were observed between control and test groups for M1 at T1 (P = 0.0385), T2 (P = 0.0029) and T3 (P = 0.0180) with higher values for control group.

Conclusions: The swimming training protocol used did not improve the athletic performance of selected horses.

Ethical Animal Research: Study approved by the Ethics Committee for Animal Use at Universidade Vila Velha (CEUA-UVV), Protocol 251-2013. **Sources of funding:** UVV-ES. **Competing interests:** none.

COMPARISON OF CUMULATIVE EFFECTS OF FOUR DAYS OF ALTERNATE OR CONTINUOUS TRAINING ON EXPRESSION OF SELECTED MITOCHONDRIAL GENES IN RAT SKELETAL MUSCLE

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Introduction: Adaptations to training include the accumulation of specific proteins. The ideal stimulus that alters gene transcriptive response that initiates changes in protein concentration is important for adaptations.

Methods: Fifty-four male Sprague-Dawley rats were randomly divided into 9 experimental groups, with six rats in each group. The groups consisted of: control (group 1); one day of training (2); continuous training groups who trained for a further two to four days followed by three days recovery (3,4,5,6); alternate-day training consisting of a further two to four sessions (7,8,9) finishing on the 7th day. On each training day, the animals ran on a motor driven treadmill at a slope of 10° and a speed of 19.3 m/min (~76%VO_{2max}) for 90 minutes. Gastrocnemius muscle specimens were obtained from the respective groups 1 hour after training ceased. Real-time PCR was performed to determine the changes (in fold) in mRNA of PGC1α, COXIV, CaMKIV, NRF1, AMPKα1 and α2, and mtTFA. One-way ANOVA was used in comparisons of the mean values between groups.

Results: The results showed that mRNA expression was significantly different between the training groups for AMPkα1 after the 3rd training session, PGC1α after the 4th training session, with CaMKIV significantly different following three days of recovery. COXIV and mtTFA were not significantly different at any point.

Conclusions: Training design has differing effects on expression of the selected genes. This data has provided insight into the importance of training design and recovery intervals which warrants a need for further research.

Ethical Animal Research: The study was approved by the Southern Cross University Animal Care and Ethics Committee. **Sources of funding:** None declared. **Competing interests:** none.

THE INNATE IMMUNE RESPONSE IN THE AIRWAYS AND IN BLOOD IS ALTERED BY TRAINING IN HORSES

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Introduction: Lower airway diseases are common problems in sports and racing horses. In humans, exercise causes increased susceptibility to respiratory infections associated with down-regulated expression of Toll-like receptors (TLRs), co-stimulatory and antigen-presenting molecules. Because numerous cell types are implicated in important defense mechanisms against airborne infections, we aimed to assess the effect of training on innate immune responses of pulmonary alveolar macrophages (PAM), equine bronchial epithelial cells (EBEC) and blood monocytes.

Methods: PAM, EBEC and monocytes were collected at rest and after a standardized exercise test (SET) from eight untrained, trained and deconditioned horses. We compared their respective expression of TLR1-9 by qPCR and their cytokinic responses following exposure to various TLR ligands by ELISA. Data were reported as least square means and analyzed by a global linear mixed model.

Results: The mRNA expression of TLR3 was down-regulated by training in PAM (P<0.0001) and monocytes (P<0.015), whereas the mRNA expression of TLR4 was up-regulated in monocytes (P = 0.0004) only. Monocytes stimulated with TLR2- and TLR4-ligands showed increased cytokine secretion after training and deconditioning (P<0.025). EBEC from trained horses produced less TNF-alpha (P<0.05) after treatment with ligands of TLR2 and TLR3 compared with EBEC from untrained horses. The production of TNF-alpha (P<0.001) and IFN-beta (P<0.001) in non-stimulated and stimulated PAM was decreased in trained and deconditioned horses.

Conclusions: The impaired cytokine production in PAM and EBEC after a training period might explain the increased susceptibility to respiratory viral infections and is associated with the activation of cells at the systemic level.

Ethical Animal Research: This work was approved by the Animal Ethics Committee of the National Veterinary School of Alfort (agreement number: 13/12/11-9). **Sources of funding:** Hippolia Foundation, the Conseil Regional de Basse-Normandie, the European Regional Development Fund and the Fonds unique interministeriel. **Competing interests:** none.

A QUESTIONNAIRE ON TRAINING OF THE ICELANDIC PACE HORSE

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Introduction: The Icelandic horse is popular and can be found in more than 30 countries. The breed is used for a variety of competitions but mounted pace competitions have the longest history. Despite this, pace training strategies have not been well documented. The aim of this study was to describe training strategies used by experienced trainers of Icelandic pace horses.

Methods: The study is based on the response to a questionnaire answered by nine trainers (age 42 ± 13 years and 23 ± 12 years of experience as pace trainers). The trainers selected were living in Iceland or Sweden, were on the world ranking list in 250 m pace races (FEIF, August 2013) and had a minimum of 12 years of experience in pace training. The questionnaire was sent to the trainers by e-mail and answers were obtained in text or during a phone interview. The questionnaire included open questions about training strategies, the development of the horse and physical health problems. Data is presented as mean ± SD.

Results: Training commenced at an age of 5.4 ± 1.0 years. Horses were trained five times per week (4.8 ± 0.7) and pace training was performed less than twice per week (1.6 ± 0.9). The average time to achieve competitive condition was 1.9 ± 0.8 years. Horses were considered to be at their best at 13.8 ± 4.6 years of age. Wounds after cross-firing (n = 3) and reactions within the suspensory ligament (n = 2) were the most common health problems mentioned.

Conclusions: In Icelandic pace horses, training appears to commence at mature age and although they are trained most week days, training in pace is limited.

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Ethical Animal Research: No animals were used in the study. **Sources of funding:** Holar University College, Iceland and the Swedish National Trotting School in Wången, Alsen, Sweden. **Competing interests:** none.

HEART RATE RECOVERY TIME IN EXERCISE TESTING OF ENDURANCE HORSES

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Introduction: Endurance horses must attain heart rates below a certain limit (usually 64 beats/min) to pass veterinary inspections in endurance rides. The time necessary for heart rate recovery is therefore important in this equestrian discipline. The objective was to determine heart rate (HR) and heart rate recovery time (HRtime) of endurance horses exercised at various velocities.

Methods: Twelve Arabian endurance horses completed an incremental field exercise test with concurrent ECG-recording. Horses were actively competing at distances of 80–160 km. The exercise protocol defined five intervals with intended velocity range of 11–13 km/h, 15–17 km/h, 18–20 km/h, 21–25 km/h, and >25 km/h, respectively. Each interval was terminated by a halt lasting until HR declined to 64 beats/min. Velocity was determined by a global positioning system, while HR and HRtime were calculated from the ECG. Descriptive statistics was performed.

Results: Velocity, HR and HRtime (as mean ± SD); Interval 1: 12.4 ± 0.9 km/h, 104 ± 11 beats/min, 50 ± 34 s; Interval 2: 15.9 ± 0.5 km/h, 120 ± 9 beats/min, 128 ± 81 s; Interval 3: 20.0 ± 0.6 km/h, 133 ± 10 beats/min, 202 ± 120 s; Interval 4: 22.9 ± 1.0 km/h, 140 ± 11 beats/min, 264 ± 170 s; and Interval 5: 29.3 ± 3.8 km/h, 171 ± 15 beats/min, 341 ± 187 s. HR showed linear correlation with velocity. HRtime varied between horses especially after greater velocities, with a tendency of being shortest in the best performing horses. Four outliers of HRtime were excluded from horses reluctant to stand still during the halt due to anticipatory excitement.

Conclusions: Heart rate recovery time shows great individuality and could potentially be used as a performance indicator in endurance horses. Possible fluctuations of HR due to anticipatory excitement during the halt may prolong HRtime and must be accounted for.

Ethical Animal Research: Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** University of Copenhagen. **Competing interests:** None.

FIELD SURVEY ON FETLOCK PROBLEMS IN YOUNG HORSES

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Introduction: Problems in the musculoskeletal system are the most common reason for impaired function and culling in insured riding horses in Sweden. Impaired function of the fetlock, e.g. arthritis, is a believed to be a multi-factorial problem. The aim of the pilot study was to find factors associated with problems in the fetlock.

Methods: This study focused on horses 5–7 years with fetlock problems. A questionnaire on training and management (feeding, housing, shoeing) was sent to 200 horse-owners with insurance policies on their horses; 100 had claimed insurance compensation for problems in the fetlock (I-horses) and 100 owners had healthy horses that served as controls (C-horses). The results of the proportions in each group were compared with a Chi-squared test. The response rate was 42%.

Results: The results showed significant differences for training-related factors but not for management-related factors. The healthy C-horses were ridden for a longer period of time at each occasion (58.5 minutes per day) compared to I-horses (51.3 minutes per day) (P = 0.043). The C-horses jumped once a week and the I-horses 0.5 times per week (P = 0.0075). There was a tendency for shorter period of training free weeks to be beneficial (C-horses 4.0 weeks, I-horses 5.5 weeks per year; P = 0.083). A larger proportion of the I-horses participated in the Swedish Riding Horse Quality Test for four-year old horses (P = 0.043).

Conclusions: The results indicated that training-related factors were associated with whether a horse remained healthy or not. Further studies on training and educational systems of young horses are needed to prevent impaired function.

Ethical Animal Research: No animals were used in the study. **Sources of funding:** This study was financed by the insurance company 'Agria' which approved submission of the results for presentation and publication in conjunction with ICEEP 9. **Competing interests:** none.

EFFECTS OF DIFFERENT EXERCISE INTENSITIES DURING DETRAINING ON AEROBIC CAPACITY IN THOROUGHBRED HORSES

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Introduction: Thoroughbred racehorses often experience periods of detraining due to locomotor disorders and subsequent rehabilitation. We hypothesised that regularly exercising horses at different intensities during a period of detraining would differentially maintain their cardiopulmonary and O_2 transport capacities.

Methods: Twenty-seven Thoroughbred horses trained for 18 weeks then underwent 12 weeks of detraining according to one of three protocols: 1) cantering at 70% maximal rate of O_2 consumption (VO_{2max}) for 3 min/d for 5 d/wk (C group), 2) walking for 1 h/d for 5 d/wk (W group) and 3) stall rest (S group). Standardised treadmill exercise tests were performed before and after detraining during which cardiopulmonary and O_2 transport variables were measured. Data were analysed with two-way ANOVA and pairwise comparisons made with Tukey's test. Significance was defined as $P \leq 0.05$.

Results: Mass-specific VO_{2max}, maximal cardiac output and maximal stroke volume of all groups decreased after detraining with no differences between groups. The run time to exhaustion, speed eliciting VO_{2max}, speed eliciting maximal heart rate and speed at which plasma lactate concentration reached 4 mmol/l of the C group did not change but those of the W and S groups decreased after detraining. Arterial-(mixed-venous) O_2 concentration difference did not change after detraining in any group, and maximal heart rate decreased in the W and S groups.

Conclusions: These results suggest it may be possible to identify a minimum threshold exercise intensity or protocol during detraining in horses that maintains significant elements of performance-related variables and minimises reductions in O_2 transport capacity.

Ethical Animal Research: Protocols for the study were approved by the Animal Welfare and Ethics Committee of the Japan Racing Association (JRA) Equine Research Institute. **Sources of funding:** Japan Racing Association. **Competing interests:** none.

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TRAINING OF HORSES USED FOR SHOW JUMPING AND ITS EFFECT ON VLa4

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Introduction: There are few data on training horses used for show jumping (SJ) in practice. This study presents such data and relates it to changes of VLa4 during an outdoors season (VLa4 = speed at which a blood lactate concentration of 4 mmol/l is achieved).

Methods: The training of 19 SJ (11 managed by professional trainer, PSJ, and 8 by amateur riders, ASJ) was documented and related to changes of VLa4. All horses were submitted to 3 standardized exercise tests (SET) to calculate VLa4: April/May, June/July, September: SET1, SET2, SET3. Noted daily were: Minutes of turn-out on pasture, of walking in horse-walker, of lungeing and of specific exercise (gymnastics, jumping and others). Data were analyzed by repeated measures ANOVA (Fisher's test as applicable) or linear regression.

Results: The duration of the specific exercises ranged between 30–60 minutes. PSJ were exercised less often and duration of each specific exercise was lower than for ASJ ($P = 0.01$). Considering all horses, the VLa4 increased between SET1 and SET2 when the duration of exercise and gymnastics was longer and the number of days on pasture higher ($P<0.05$ both). These relationships became not significant when groups were analysed separately. The VLa4 of ASJ increased between SET2 and SET3 when horses were kept on pasture for more time ($P<0.05$, $r2 = 0.64$). The VLa4 of PSJ tended to increase with a higher percentage of jumping days between SET1 and SET2 ($P = 0.08$, $r2 = 0.30$), and with longer duration of jumping exercise sessions between SET2 and SET3 ($P = 0.06$; $r2 = 0.33$).

Conclusions: The information may help to improve the training of show jumpers.

Ethical Animal Research: Horse owners gave consent for these procedures within a veterinary monitoring system. **Sources of funding:** none declared. **Competing interests:** none.

YOUNG FRIESIAN HORSES SHOW FAMILIAL AGGREGATION IN FITNESS RESPONSE TO A 7-WEEK PERFORMANCE TEST

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Introduction: The aims of the study were to monitor fitness level of young Friesian horses and to assess whether fitness data has a predictive value for the final performance score and whether familial aggregation of response to training could be detected.

Methods: Sixty-six young Friesian horses, offspring of six different stallions (A, B, C, D, E and F), underwent a 7-week performance test. Horses were given a performance score for their dressage ability (0–110 points), and fitness was evaluated with standardised-exercise-tests (SET) at the beginning (SET-I) and the end (SET-II) of the period. Heart rate (HR; beats/min) was measured in both SET, and plasma lactate concentration (LA; mmol/L) was measured only in SET-II. The majority of data were analyzed with an ANOVA-test or a linear-model ($P<0.05$).

Results: Fitness of horses improved significantly between SET-I and II ($P = 0.015$). There was a large heterogeneity in responsiveness to training; some horses were high-responders, whereas others were non- or low-responders. There was a familial aggregation of HRcanter-1 response to training ($P = 0.039$); offspring of stallion C decreased significantly more in HR than offspring of stallions A ($P = 0.09$), D ($P = 0.013$) and F ($P = 0.009$). HR had no predictive value for the performance score, but horses that did not reach the anaerobic threshold in SET-II scored significantly better (73.8 ± 5.6 points) than horses that did (69.9 ± 5.9 points, $P = 0.025$).

Conclusions: The present study reports, for the first time in the horse, familial aggregation of HR-response to training. HR could not predict final performance score, but LA-concentrations during SET-II were predictive.

Ethical Animal Research: Consent was obtained from the owners of all horses. **Sources of funding:** partly financially supported by the Royal Friesian Horse Studbook (KFPS). **Competing interests:** none.

SUBJECTIVE EVALUATION OF MUSCLE CONFORMATION IN HORSES SUBJECTED TO PACE TRAINING OR NOT

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Introduction: Muscle conformation is often discussed among practitioners and it is sometimes suggested that specific training strategies affect muscle conformation. This study attempted to investigate; 1) if it is possible for practitioners to judge whether a horse is subjected to pace training or not based on subjective analysis of hind-end muscle conformation and 2) which terminology practitioners would use when doing this.

Methods: Photos were taken from behind (transverse plane) in 10 Icelandic horses trained for mounted pace racing and 11 used for riding but not pacing. Body condition score was 7.4 and 7.5 (SE 0.3, $P>0.05$) in pacers and non-pacers, respectively (9 degree scale, ANOVA, Tukey test). Three experienced horse practitioners (A, B, C) were informed that the 21 photos showed horses trained for pace racing and horses not trained as pacers and they were instructed to separate photos into two groups based on their subjective impression of muscle conformation.

Results: Practitioner A divided horses into groups called "even" ($n = 13$) and "narrow" ($n = 8$); B into groups called "strong" ($n = 11$) and "weak" ($n = 10$); and C into "high" ($n = 9$) and "wedged" " ($n = 12$). The practitioners assumed that "even", "strong" and "high" horses were pacers and 10, 10 and 12 observations were correct in practitioner A, B and C, respectively (mean ± SD: 51 ± 5%). The number of cases where all practitioners agreed and grouping also matched pacers and non-pacers were 7.

Conclusions: It appears not to be possible to judge whether an Icelandic horse is pace trained based on observations of the hind-end musculature. The terminology used appeared to refer either to the conformation or to a presumed strength.

Ethical Animal Research: Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Holar University College, Iceland. **Competing interests:** none.

TRAINING PROTOCOL FOR MARCHA TEST IN MANGALARGA MARCHADOR HORSES

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Introduction: Mangalarga Marchador (MM) is the most important Brazilian horse breed. Functional evaluation of MM horses is performed in

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marcha competitions, where horses must perform 4-gaited exercise at 13 km/h for 70 min. The aim of this work was to test a training protocol to prepare MM horses for marcha competition.

Methods: Eight untrained MM mares, in randomized blocks with Split plots, were trained for 84 days. Three days/week horses were ridden for one hour (10 min walk, then an individual amount of marcha exercise at 13 km/h established in a previous test, then walk to complete one hour total exercise time). Two days/week, horses walked for one hour. Before training and every 21 days a marcha test (MT) was performed, totaling 5 tests in the experimental period. During the MT, mares were exercised at 13 km/h using a heart rate monitor. Every 10 min heart rate (HR) and blood lactate concentration [La] were recorded. The MT was stopped at 70 min of exercise, or if HR was ≥150 bpm and [La] was ≥4 mmol/L, or if Marcha Time was reached. Blood samples for hemogram, lactate, glucose, triglyceride and CK, LDH, AST determination were collected. Individual time used for training was 10 min less than the exercising time in the previous MT. Regression was used to analyze the results.

Results: From MT 1 to 4, the positive effect of training was shown on HR, [La] and marcha time (from 9.52 min to 70.5 min, CV = 26.41). There was not an increase in marcha time on test 5.

Conclusions: MM mares trained for 63 days with the described protocol are ready to compete in marcha competition.

Ethical Animal Research: The study was approved by the Ethical Animal (CETEA) of the UFMG with the 255/11 protocol. **Sources of funding:** CNPq, CAPES, FAPEMIG, ABCCMM, Haras Catuni e Rações Total. **Competing interests:** none.

EFFECTS OF AMOUNT OF INTENSIVE TRAINING ON VLa4 IN 3-YEAR OLD STANDARDBRED HORSES

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Introduction: The effects of different amounts of training on performance of young horses have not yet been studied in a long term perspective. The aim of this study was therefore to assess the effect on VLa4 of two training programs performed from the age of 2 to 3 in Standardbred horses.

Methods: Fourteen horses were in March as 2-year olds divided into two groups; one was randomized to a training program including intensive exercise (control) and the other to a training program with reduced amount of intensive exercise (distance –30% compared to control). Group division was partly based on breeding index (means: control 110, reduced 109) and gluteus medius fiber type ratio (IIA/IIB, both groups mean 1.34). Horses were trained ~2 times/week. After 14 months, a VLa4-test was performed on a track (4 x 1000 m). Speed was measured by GPS and increased by ~3 s/1000 m for every interval. Blood samples were collected after each interval and lactate concentration was determined using Lactate Pro. Exponential regression analysis of speed and lactate concentration were used to estimate individual VLa4. ANOVA and Tukey's test were used to analyse differences between groups.

Results: There was no difference in VLa4 between the control and reduced exercise group (10.6 ± 0.1 and 10.8 ± 0.1 m/s).

Conclusions: The study indicates that it might be possible to reduce the amount of intensive training by ≤30% without impairing performance of 3 year old Standardbred horses.

Ethical Animal Research: The study was approved by the local ethics committee in Umeå. **Sources of funding:** Swedish University of Agricultural Sciences, Swedish National Trotting School at Wången, Swedish Trotting Association, Swedish Horse Council Foundation, Swedish Horse Racing Totalisator Board (ATG), Trioplast AB, Dow Chemicals, Polar Sverige AB, and Scandivet AB. **Competing interests:** none.

THE RELATIONSHIP BETWEEN BLOOD LACTATE, SERUM MUSCLE ENZYMES, JUMPING PERFORMANCE AND MUSCLE SORENESS IN SHOW-JUMPING HORSES

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Introduction: There is relatively little information concerning physiological factors affecting show-jumping performance. This study examined the relationship between show-jumping exercise, muscle soreness, serum muscle enzyme activity and blood lactate concentration.

Methods: Ten mixed breed horses (9.1 ± 1.9 yrs) were assessed at a British Equestrian Federation (BEF) World Class Performance three-day training session. Riders received coaching during warm-up and after jumping a 15 fence course (1.35–1.45 m) once on day one and twice on day two; on day three the second round was an 8 fence jump-off. Veterinary and physiotherapy assessments of muscle soreness were performed daily before and after exercise. An international coach graded jumping technique. Blood was obtained before and on two occasions after jumping (1 minute, two hours) for measurement of serum CK and AST, and blood lactate concentration. Data were analysed using regression procedures or Spearman correlation, and Wilcoxon signed-rank test ($P<0.05$).

Results: Blood lactate significantly increased one minute after jumping on all three days ($P = 0.003$ for all). When two rounds were jumped, lactate was significantly lower after the second round compared to the first ($P = 0.006$, $P = 0.014$). There was a positive correlation between blood lactate one minute after jumping and number of faults scored daily and overall ($R2 = 0.61$; $P<0.01$), decreased jumping technique grade ($R2 = 0.69$; $P<0.01$) and increased gluteal muscle soreness on day two ($r = 0.75$; $P = 0.012$). Plasma CK activity one minute, and 2 hours after jumping was significantly higher than before jumping on all days ($P<0.01$).

Conclusions: Horses with higher blood lactate concentrations after jumping demonstrated decreased jumping performance and were more likely to display muscle soreness.

Ethical Animal Research: Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** The World Class Development Programme is funded by the Department for Culture, Media and Sport, through Exchequer and National Lottery funds, via UK Sport. **Competing interests:** none.

EFFECT OF CONDITIONING HORSES WITH RUNS OF 100 METERS AT NEAR MAXIMAL SPEED ALONE OR IN COMBINATION WITH LOW INTENSITY EXERCISE

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Introduction: Exercising horses one, two, or three times per week during 6 weeks using two intervals with near-maximal speed over 100 m elicited a decrease of VLa4, a widely accepted parameter of fitness and competitive success of horses (speed at which, under defined conditions, the blood lactate concentration reaches 4 mmol/l). Therefore, two field studies were performed to examine the possibility of avoiding decline in VLa4 with this type of exercise.

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Methods: Study 1) 6 horses were submitted to the same exercise as above once per week during the first 2 weeks of a conditioning period (CP) adding one interval in each exercise session every 2 weeks for the following 6 weeks. Study 2) 6 horses did the same as horses in study 1 plus once per week an exercise bout at their individual VLa2 during 35 minutes. Before, every 2 weeks during, and 2 weeks after the CP, horses were subjected to standardized exercise tests to determine their VLa4 (and VLa2 where convenient) and maximal speed (speedmax). The effect of conditioning on VLa4 and speedmax was examined using two-way repeated measures analysis of variance and Fisher's test.

Results: In study 1 mean VLa4 declined during the first 4 weeks of CP but increased to the initial level thereafter ($P<0.05$) while speedmax remained the same over time. In study 2 VLa4 decreased continuously ($P<0.01$) while speedmax increased ($P<0.01$).

Conclusions: The results might be important to train horses racing over different distances.

Ethical Animal Research: All procedures were approved by the Bioethical Committee of the University Nacional del Litoral, Esperanza, Argentina. **Sources of funding:** Verein zur Förderung der Forschung im Pferdesport, Germany, AVIGAN Alimentos Balanceados and Pellfood. **Competing interests:** none.

PREDICTIVE VALUE OF FITNESS TESTS AND REASONS FOR WITHDRAWAL IN HORSES/PONIES SELECTED FOR THE EUROPEAN EVENTING CHAMPIONSHIP 2010–2011

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Introduction: Eventing is a challenging equestrian discipline. Insight into causes of wastage and withdrawal from competition is essential for animal welfare and economic reasons.

Methods: Twenty-nine animals selected for the European Championship (EC) in 2010 (9 ponies) and 2011 (20 horses) were followed prospectively monitoring fitness using standardized exercise tests. Causes of withdrawal were noted. Data were analyzed using logistic-regression, likely-ratio-tests, Fisher-exact-test, general-linear-model and Chi-square tests as appropriate.

Results: Seventeen weeks before EC 17 horses and 9 ponies were tested: mean VLa4 was 10.3 ± 0.4 m/s and 7.8 ± 0.9 m/s respectively, and V200 was 11.4 ± 0.8 m/s and 9.6 ± 0.7 m/s respectively. Six weeks before EC 16/20 horses and 6/9 ponies were withdrawn, mainly for locomotor injury: 9/16 horses ($P<0.001$), 4/6 ponies ($P = 0.011$). Other reasons were 'not-meeting-competition-criteria' (4/16 horses, 2/6 ponies) and 'being sold' (3/16 horses). Divided on the basis of VLa4 and 'recovery-HR-during-SET-I' into good (GP) and average performers (AP), AP were more likely to be injured (50%) than GP (0%, $P = 0.05$). In a subpopulation ($n = 10$, all condition training sessions evaluated for HR and speed) HRpeak was significantly lower in horses staying sound (186 ± 9 beats/min) compared to horses withdrawn because of injury (201 ± 5 beats/min; $P = 0.016$).

Conclusions: For the European Championship 45% of national selection horses were unavailable because of locomotor injuries. GP at the beginning of the season were less likely to become injured than AP. HRpeak during condition training showed predictive value for future injuries.

Ethical Animal Research: Owners gave consent for horses to participate in the study. **Sources of funding:** Faculty of Veterinary Medicine and Dutch National Equestrian Federation. **Competing interests:** none.

EFFECTS OF AGE AND EXERCISE TRAINING ON OXIDATION OF SKELETAL MUSCLE AND BLOOD IN MATURE AND YEARLING HORSES

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Introduction: The objective of this study was to determine if yearlings would have higher oxidative stress and lower antioxidant status than mature mares after a single bout of intense exercise before and after training.

Methods: Seven Standardbred yearling fillies (18 ± 2.4 mo) and seven mares (13 ± 2.1 yr) ran a repeated sprints exercise test before (RSET1) and after (RSET2) an 8-wk training protocol. Blood samples and middle gluteal muscle samples were taken before and after exercise, and throughout recovery. Data is presented as mean ± SE and all analyses were done in the context of a linear mixed model with random intercepts for horse.

Results: In RSET1, mares had higher plasma malondialdehyde than the yearlings ($P<0.05$), but the yearlings had higher muscle malondialdehyde, nitric oxide, glutathione and glutathione peroxidase, erythrocyte total glutathione and glutathione peroxidase ($P<0.05$). In RSET2, yearlings had higher plasma nitric oxide than the mares ($P<0.05$). In RSET2, the mares had lower plasma malondialdehyde, and higher muscle malondialdehyde, total glutathione and nitric oxide as compared to RSET1 ($P<0.05$). The yearlings in RSET2 had higher muscle nitric oxide and lower erythrocyte glutathione peroxidase than in RSET1 ($P<0.05$). Plasma cortisol, creatine kinase and muscle glutathione peroxidase had little difference with age or training.

Conclusions: Prior to training, the yearlings had lower lipid peroxidation and higher antioxidants than the mares. Training reduced oxidative stress and improved antioxidant status in the mares, while few effects of training were seen in the yearlings. This suggests that age is an important defense against exercise-induced oxidative stress.

Ethical Animal Research: Rutgers University Institutional Animal Care and Use Review Board approved the study. **Sources of funding:** none. **Competing interests:** none.

QUANTIFYING WARM-UP IN SHOWJUMPING HORSES OVER 3 CONSECUTIVE DAYS

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Introduction: The objective was to quantify warm-up (WU) jumping patterns/duration in showjumping horses.

Methods: Ten mixed breed horses (9.1 ± 1.9 yrs) were assessed at a British Equestrian Federation (BEF) World Class Performance three day training session. Riders received coaching during WU and after jumping a 15 fence (1.35–1.45 m) course once on day-1 and twice on day-2; on day-3 the second round was an 8 fence jump-off. Fence type/height, number of jumping efforts (JE) and lead take-off/landing limbs during WU were recorded. Rider global-positioning-system recorded speed. Inertial motion sensors fixed to the lateral aspect of metatarsal/metacarpal bones, recorded time spent in each pace/rein plus stride length (SL) during WU and course. Paired Students T-tests were used to compare variables between days, as well as WU and course.

Results: Mean WU duration, time in each pace and on each rein did not differ within rider between days, however there were inter-rider differences (mean WU duration = 18 mins; range = 12–27 mins). Number

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of JE and fence type/height did not differ between days. During WU, there was marked canter lead, jump landing and leaving lead limb asymmetry (left canter lead predominating in all cases). There was no preference in canter lead when approaching WU fences. Horses cantered slower and with a shorter SL during WU compared to when jumping the course (speed-WU:4.21 ± 0.09 m/sec; course:5.53 ± 0.15 m/sec; SL- WU:2.59 ± 0.06 m; course:3.16 ± 0.08 m).

Conclusions: Results provide preliminary information on WU duration, and suggest that showjumping horses may WU asymmetrically, predominately on the left rein, at competitions and during training. This could have implications for injury risk to a particular forelimb or hindlimb in showjumping horses.

Ethical Animal Research: Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** The World Class Development Programme is funded by the Department for Culture, Media and Sport, through Exchequer and National Lottery funds, via UK Sport. **Competing interests:** none.

APPLIED PHYSIOLOGY: OTHER TOPICS

FATALITIES ASSOCIATED WITH AMERICAN ENDURANCE RIDE CONFERENCE SANCTIONED ENDURANCE RIDES (2002–2013)

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Introduction: Fatalities are recognized as a consequence of prolonged endurance exercise but data documenting incidence and causes is limited.

Methods: Numbers of horse starts and fatalities were obtained from the American Endurance Ride Conference (AERC) website (www.aerc.org) and further details about fatalities were obtained from horse owners and attending veterinarians. Annual incidence rates were calculated and odds ratios for region and ride length were determined.

Results: From 2002–2013 there were 252,738 horse starts in AERC sanctioned rides and 67 fatalities attributable to the demands of endurance exercise were documented. 46/67 horses were eliminated while 21 successfully completed the ride and were deemed fit to continue. Thirteen horses died while euthanasia was performed in the remaining 54 instances, and 51% had necropsy examinations performed. 81% of the fatalities developed acute abdominal pain and necropsy findings included gastric rupture in 12%. Fatalities were also related to financial constraints as 28% of riders declined fluid therapy, referral to surgical facilities, and/or surgery when recommended. Horses competing in the Central and Midwest regions appeared at lesser risk, while those in the Southwest and Northeast regions appeared at greater risk, but low numbers of fatalities precluded reaching statistical significance. In contrast, the risk of fatality was 2 (1 fatality per 3,711 starts) and 12 (1 fatality per 648 starts) times more likely for participation in 80 and 160 km rides, as compared to competition in Limited Distance (40 to 56 km) rides (1 fatality per 8,057 starts).

Conclusions: Overall fatality rates were low; however, risk increased with ride distance. Acute abdominal pain was the most common clinical presentation.

Ethical Animal Research: This retrospective study used data available on the American Endurance Ride Conference (AERC) website and additional documents provided by the AERC. **Sources of funding:** none. **Competing interests:** Dr. Balch is a member of AERC Veterinary Committee, Research Committee, and Board of Directors and Dr. Schott is a member of the AERC Research Committee.

TIMING OF BLOOD SAMPLING FOR POOR PERFORMANCE DIAGNOSIS IN STANDARDBRED HORSES AFTER RACING

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Introduction: Blood is often collected from performing poorly horses to measure blood variables that may indicate a reason. However, intense exercise affects many blood variables and therefore time of sampling can affect the interpretation of results. This study examined the behaviour of blood variables after racing to determine the magnitude of the changes and to determine when it is better to collect samples.

Methods: Blood was sampled in 17 Standardbred racehorses 3 and 2 days before as well as 2 and 3 days after racing. GGT, albumin, cholesterol, creatinine, Ca, P, Mg, Na, K, CK, AST, LDH and cortisol were measured in plasma, and haematocrit, haemoglobin, number of erythrocytes, total white blood cells, granulocytes and lymphocytes were measured in EDTA blood. Parametric data was analyzed with ANOVA for repeated measures and Fisher test; non-parametric data with Friedman test and Wilcoxon test.

Results: Plasma Ca concentration was lower on day 3 after racing than on day 2 before racing ($P<0.01$). P concentration and GGT activity were higher at days 2 and 3 after racing compared to before racing ($P = 0.01$). Erythrocytes, haematocrit and haemoglobin were higher at day 2 after racing than before and 3 days after racing ($P<0.01$). The other variables were not affected.

Conclusions: Some slight but significant changes in blood variables have to be taken in account when interpreting biochemical and haematologic variables for poor performance diagnosis in Standardbreds. It seems preferable to take blood samples on the third day after racing than on the second day.

Ethical Animal Research: Horse owners gave consent for these procedures within a veterinary monitoring system. **Sources of funding:** Verein zur Förderung der Forschung im Pferdesport and Laboklin, Germany. **Competing interests:** none.

INNATE VARIABILITY IN VOLUNTARY DRINKING IN ARABIAN HORSES DURING ENDURANCE EXERCISE

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Introduction: Human athletes have been characterized as either 'good drinkers' or 'poor drinkers' on the basis of voluntary water intake during and after endurance exercise. Similarly, riders of endurance horses have described some horses to be better drinkers than others; however, there are no data in horses to substantiate these claims.

Methods: Voluntary fluid intake (FI) by twenty four 2-year-old Arabian horses that performed 45–60 km of treadmill exercise in four experiments was recorded. All horses were raised similarly and had no training except a 12 week conditioning period before each experiment. Mean FI intake (each

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horse performed three or four exercise tests in each experiment) was subjected to cluster analysis to separate horses into poor, average, and excellent drinking groups. FI was also correlated to body weight (BW) loss at the end of the exercise test.

Results: Cluster analysis separated horses into poor drinkers (n = 10, FI 8.7 ± 0.7 liters and BW loss of 2.2 ± 0.4%); average drinkers (n = 11, FI 14.1 ± 0.6 liters and BW loss of 1.8 ± 0.2%); and excellent drinkers (n = 3, FI 24.9 ± 0.2 liters and BW loss of 0.7 ± 0.3%). FI was inversely correlated with BW loss (R values between –0.53 and –0.87 for all four experiments, P<0.01).

Conclusions: These data, in groups of young, similarly trained horses, are the first to support that there are innate differences in voluntary drinking during and after endurance exercise in this species.

Ethical Animal Research: All procedures were approved by the Animal Use Committee of Michigan State University **Sources of funding:** Equine Health Research Fund of the American Horse Shows Association, WALTHAM Centre for Pet Nutrition, and the Department of Large Animal Clinical Sciences, Michigan State University. **Competing interests:** none.

THE IMPACT OF PLAYING STRATEGIES ON RECOVERY IN LOW-GOAL POLO-PONIES

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Introduction: Polo is a popular sport; teams compete in matches comprising 4–6 chukkas, each between 7–10 minutes. Players commonly change ponies between chukkas, but changing within a chukka, known as 'half-chukkering' is permitted. This is believed to 'save' the horse by preventing fatigue and facilitating recovery. This study evaluated the impact of half versus full chukka playing strategies in low goal polo; team handicap 0–4 goals.

Methods: Resting heart rates (HR) were established for 48 polo-ponies deemed of comparable fitness by riders, competing in low-goal tournaments; riders selected their playing strategy either full-chukka (n = 32) or half-chukkering (n = 16). HR was recorded at the end of play and during recovery; for the full-chukka and 2nd half-chukka period at +2, 4, 6, 10 and 20 minutes, and until ponies returned to play after the 1st half-chukka, >6 minutes. ANOVA analyses investigated HR variation during recovery between ponies playing full-chukkas and the 2nd half-chukka period, and within the two half-chukkas played.

Results: At the end of play, full-chukka horses' mean HR were 95 ± 13.1 bpm, compared to their 2nd half-chukka counterparts at 103 ± 7.7 bpm, which was not significant. Significant differences in HR did occur throughout the subsequent recovery period: at 2 minutes (P<0.003) and between 4–20 minutes (P<0.0001), with full-chukka ponies recovering faster. No differences occurred during recovery between the two half-chukka periods although mean HR differed at the end of play (1st: 100 ± 7.7 bpm; 2nd: 103 ± 7.8 bpm).

Conclusions: Playing strategies influence cardiovascular recovery; full-chukka ponies recover faster than those playing in consecutive half-chukkas. Preparation of half-chukka ponies may predispose to reduced fitness levels compared to full-chukka ponies. Implementing a full-chukka playing strategy is advocated to optimise pony welfare and performance in low-goal polo.

Ethical Animal Research: Ethical approval for the study was granted from the UWE Hartpury Ethics Committee. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** none. **Competing interests:** none.

QUANTIFICATION OF ENERGY EXPENDITURE IN FIELD CONDITIONS: COMPARISON OF DIRECT VO_2 VERSUS VO_2 ESTIMATION FROM HEART RATE MEASUREMENTS

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Introduction: In humans, oxygen consumption (VO_2) and heart rate (HR) are commonly used to assess energy expenditure (EE) during exercise in field conditions. We hypothesized that the estimation of EE from HR measurements could be a relevant parameter to estimate EE during submaximal field exercise. This study aimed to compare the EE from VO_2 and HR measurements in exercising trotters.

Methods: Six harnessed French Trotters were submitted to a continuous field incremental test (FIT) at trot to establish their individual VO_2/HR relationship. Then they undertook a submaximal exercise bout (EX) over 2375 m during 3.41 ± 0.47 min at 70% of maximal velocity. VO_2 and HR were recorded continuously (equine K4b^2, COSMED, Rome, Italy) throughout FIT and EX. EE of EX was calculated by multiplying the total VO_2 (Liters), either directly measured or predicted from HR, with its caloric equivalent (4.86 kcal/liter). Means were compared using a Student's t test.

Results: EE of EX was estimated at 2422 ± 390 kcal and 2630 ± 706 kcal from direct VO_2 and HR methods respectively (P = 0.54).

Conclusions: We found no significant difference between the two methods for estimating EE of submaximal exercise. This suggests that the prediction of EE from HR monitoring might be used to evaluate EE of moderate intensity workouts.

Ethical Animal Research: The project was conducted under the ethics committee approval B1110. **Sources of funding:** INRA PHASE, the Burgundy Region and AgroSup Dijon. **Competing interests:** none.

EFFECT OF SHOWERS AND BLANKETS AFTER EXERCISE ON HEAT DISSIPATION IN SWEDISH STANDARDBRED TROTTERS

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Introduction: Swedish Standardbred trotters are usually not clipped during the cold season but routinely showered and blanketed after exercise. Yet, the effects on heat dissipation have not been thoroughly studied.

Methods: Six Standardbred geldings were tested during three consecutive days, acting as their own control when: 1) showered, 2) showered and blanketed, 3) neither showered nor blanketed. The exercise consisted of 20 min trot (maximum heart rate 172 bpm), preceded and followed by 5 min walk. Rectal- (RT) and skin temperature (ST), respiration rate (RR) and heart rate (HR) were recorded: before exercise in the horses' boxes, after harnessing, after 5 min walk, at stops during four sequences of low and high intensity trotting, after 5 min walk (stable aisle), after showering and blanketing and at intervals of 15–30 min during recovery in boxes until one hour after exercise. Blankets remained on horses until test completion.

Results: Data were analyzed with Proc Mixed Model in SAS. Ambient temperature was 7.2 ± 0.2°C (relative humidity 63.5 ± 1.0%) in the stable and 1.0 ± 1.0°C (54.3 ± 1.2%) outdoors. Showering decreased ST of the neck (mean ± SE, 32.0 ± 0.4°C) compared to when horses were not showered (35.1 ± 0.4, P<0.05). Showering also decreased ST of the hind leg (31.4 ± 0.5, P<0.05). RT, RR and HR did not differ between treatments (P>0.05).

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Conclusions: In conclusion, showering is efficient for cooling down whereas blanketing compromised heat dissipation.

Ethical Animal Research: This study was approved by the Local Ethics Committee (Uppsala, Ref C99/12). **Sources of funding:** Stiftelsen Hästforskning. **Competing interests:** none.

INFLUENCE OF EXERCISE IN THE OCCURRENCE OF GASTRIC ULCERS IN HORSES

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Introduction: The most frequently reported causes of gastric ulcers in horses are fasting, type of feed and management, and the administration of some drugs. More recently, physical exercise has also been pointed out as an important factor in the development of gastric lesions in athletic horses. The aim of this study was to evaluate whether treadmill exercise predisposes to the occurrence of gastric ulcers in horses.

Methods: Five Arabian horses were exercised on a high-speed treadmill during twelve weeks. The animals were submitted to gastroscopy before the beginning and the end of the training period. The lesions were classified according to the method proposed by Macallister [1] that assigns scores for number and severity of injuries, with modifications to give a total score for the stomach, considering not only the presence and severity of ulcers, but also other findings as gastritis, hyperkeratosis, desquamation of the mucosa. The results obtained in the two periods were compared using the Wilcoxon test, with significance at $P<0.05$.

Results: In the first gastroscopy two animals had very low scores for lesion number and severity and three horses had no injury. In contrast, in the second evaluation, all animals had higher scores. A significant difference was observed between the two periods (before and after training) considering both methods of gastric lesion classification.

Conclusions: We concluded that Arabian horses exercised on a treadmill may develop gastric lesions. The possible influence of these lesions on athletic performance and other parameters should be considered by researchers who study equine exercise physiology.

Ethical Animal Research: This study was approved by the Ethics Committee on Animal Use (protocol number 1741). **Sources of funding:** FAPESP 2012/10886-3. **Competing interests:** none.

Reference

[1] MacAlistair, CG, Andrews, FM, Deegan, E, Rouff, W, Olovson, SG. A scoring system for gastric ulcers in the horse. Equine Vet J, 1997, 29(6), 430-3.

STANDARDIZED EXERCISE TEST IN YOUNG ENDURANCE HORSES

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Introduction: The objective of this study was to validate a field standardized exercise test (SET) adapted for young endurance horses and to evaluate the effects of age/conditioning level on speed and heart rate values.

Methods: The study involved 31 horses of Arabian origin bred specifically for endurance and aged 4 yo (n = 11), 5 yo (n = 13) or 6 yo (n = 7). The SET consisted of a warm-up, followed by 15 (4 yo), 30 (5 yo) or 45 (6 yo) minutes of canter at a comfortable steady speed, and finished with 500 meters of fast gallop. Heart rate (HR) was recorded continuously during the SET. The velocities at which HR reached 160 and 200 beats per minute (V160 and V200) were calculated by the linear regression of HR versus speed.

Results: All horses were able to complete the SET. Resting HR was higher in 4 y than in 5 y and 6 y ($P<0.05$ via Student's t test). HR and velocities during the SET did not vary significantly with age. Mean ± SD values for 4 yo, 5 yo and 6 yo respectively are as follows: Resting HR: 47.5 ± 7.2, 42.3 ± 3.2, 41.3 ± 5.4 bpm; HRcanter: 162.7 ± 14.3, 156.9 ± 17.3, 161.4 ± 10.7 bpm; Vcanter: 22.6 ± 0.9, 22.4 ± 1.2, 23.3 ± 1.6 km/h; HRsprint: 191.3 ± 18.1, 181.7 ± 18.5, 184.1 ± 16.2 bpm; Vsprint: 30.3 ± 3.0, 31.5 ± 6.4, 35.3 ± 6.9 km/h; V160: 25.8 ± 3.5, 24.8 ± 5.0, 27.1 ± 5.8 km/h; V200: 36.8 ± 6.1, 37.4 ± 7.3, 44.3 ± 10.9 km/h; Post-exercice lactatemia: 7.4 ± 4.5, 13.5 ± 3.6, 5.6 ± 3.4 mmol/l. These values perfectly fit with those described previously for adult endurance horses.

Conclusions: The proposed field SET involved both aerobic and anaerobic metabolism reproducing the progress of an endurance race. Further studies are being conducted to discriminate if possible the effects of age from those of conditioning.

Ethical Animal Research: The study was approved by the Ethics Committee for the Alfort Veterinary School and the University of Paris-Est; Owner informed consent for participation was obtained. **Sources of funding:** National Studs (IFCE), The Eperon Funds and the Arabian Horse Association (ACA). **Competing interests:** none.

CHANGES IN ABDOMINAL CIRCUMFERENCE DURING ENDURANCE EXERCISE: A PILOT STUDY

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Introduction: Horses have a large intestinal reserve of water and electrolytes that can be absorbed to replace sweat fluid losses during endurance exercise. As a consequence, the abdomen develops a "tucked-up" appearance; however, data documenting changes in abdominal circumference during endurance exercise are lacking.

Methods: Abdominal circumference was measured directly (in triplicate with a measurement tape to nearest cm) every 15-km at three sites (front, middle, and back) in six 2-year-old Arabian horses that completed four 60-km bouts of treadmill exercise. Data were analyzed by repeated measures ANOVA and compared to body weight (BW) loss by correlation analysis.

Results: Abdominal circumference decreased at all three sites during the exercise test (1.8 ± 0.7, 1.4 ± 0.7, and 3.1 ± 1.3 cm for front, middle, and back, respectively, [representing 1–2% of total circumference]) with the greatest decrease (>50% of total) occurring during the initial 15-km of the exercise test. Changes in abdominal circumference during each step of the exercise test were significantly correlated with BW changes (R values 0.18–0.39, $P<0.05$ for all).

Conclusions: These data support the clinical observation that the abdomen decreases in size during endurance exercise and that the largest changes are observed in the earlier stages of exercise, similar to the initial greater decrease in BW that has been documented in several studies during the initial half of endurance competitions.

Ethical Animal Research: This study was approved by the Animal Use Committee of Michigan State University. **Sources of funding:** WALTHAM Centre for Pet Nutrition. **Competing interests:** none.

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RELATIONSHIP BETWEEN JUDGES' COMMENTS ON PACE AND HORSES' PLASMA LACTATE CONCENTRATION IN AN OFFICIAL BREED EVALUATION FIELD TEST IN ICELANDIC HORSES

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Introduction: In an official breed evaluation field test (BEFT) for Icelandic horses, gaits are scored from 5–10 and there is an option for judges to add standardized comments on the gaits. The aim of this study was to test if comments given on pace were related to horses' plasma lactate concentration (Lac) post BEFT, with the hypothesis that some of the comments would be related to Lac.

Methods: Data was collected from 157 horses (age 4–11 years) at a BEFT in Iceland in 2011. Venous blood samples were obtained after exercise and lactate concentration determined. Judges' comments were obtained from Worldfengur. The comments that could be given were: 'good speed', 'clear beat', 'sure', 'big movements', 'good suspension', 'long strides' (positive comments); and 'lack of speed', 'unsure', 'four-beat', 'short strides', 'changing gait' and 'jumping out of gait' (negative comments). Results were separated into two groups (within each comment); horses/pace with the comment and horses/pace without the comment. Data were analysed via ANOVA (Proc GLM, SAS) including sex, age group, comment, average speed and distance ($P<0.05$).

Results: Lac was higher when pace had comments 'good speed' and 'sure' than when no comment was given (23.6 ± 1.2 vs. 17.9 ± 0.5, $P<0.001$; and 20.2 ± 0.9 vs. 18.1 ± 0.6, $P<0.05$) and was lower when pace had comments 'lack of speed' and 'unsure' (16.8 ± 1.0 vs. 19.2 ± 0.6; and 16.2 ± 1.0 vs. 19.3 ± 0.5, $P<0.05$). No other significant effects on Lac were observed.

Conclusions: Horses with pace subjectively judged as fast and 'sure' had higher blood lactate concentration indicating that successful pace is more anaerobic than less successful pace.

Ethical Animal Research: The study was approved by the National Animal Research Committee of Iceland. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Developmental Fund of the Icelandic Horse Breeding, the Pálmi Jónsson Natural Conservation Fund and the Stock Protection Fund of the Icelandic Horse breed. **Competing interests:** none.

A PILOT STUDY ON THE PHYSIOLOGICAL RESPONSE TO AN OFFICIAL BREED EVALUATION FIELD TEST IN ICELANDIC HORSES

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Introduction: The aim of this study was to describe the response in heart rate, respiratory rate, rectal temperature, hematocrit and plasma lactate concentration in Icelandic horses performing the riding assessment in an official breed evaluation field test (BEFT) in Iceland (April 2011). It was hypothesized that the riding assessment constituted high intensity exercise.

Methods: Data was collected from 15 of 28 horses shown at the BEFT. Horses were judged for riding abilities (all gaits) on a straight track. Respiratory rate and rectal temperature were recorded and blood samples were taken from the jugular vein before the warm-up and within 5 min after the riding assessment. Hematocrit was analysed by centrifugation of capillary tubes and plasma lactate concentration using an enzymatic and spectrophotometric method. Velocity, distance and heart rate were recorded (Polar, Kempele, Finland) during the riding assessment. Anova (Proc GLM) was performed in SAS and data is presented as LSMean ± SE ($P<0.05$).

Results: The distance covered was 2.7 ± 0.1 km, the duration $9{:}13 \pm 0{:}21$ min : sec and the average speed 17.5 ± 0.3 km/h. The peak heart rate was 214 ± 3 bpm. Post-exercise plasma lactate concentration was 18.6 ± 1.7 mmol/L and there was an increase ($P<0.001$) in respiratory rate (40 ± 6 to 118 ± 6 breaths/min), rectal temperature (38.1 ± 0.1 to 39.8 ± 0.1°C) and hematocrit (35 ± 2 to 47 ± 2%) following the breed evaluation field test.

Conclusions: The physiological responses indicate that the riding assessment in BEFT is a demanding exercise, requiring some contribution of anaerobic metabolism.

Ethical Animal Research: The study was approved by the National Animal Research Committee of Iceland. Owners and trainers were informed that participation was voluntary. **Sources of funding:** Developmental Fund of the Icelandic Horse Breeding, the Pálmi Jónsson Natural Conservation Fund and the Stock Protection Fund of the Icelandic Horse breed. **Competing interests:** none.

VALUE OF FIELD TRIALS TO INVESTIGATE POOR PERFORMANCE IN SPORT HORSES

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Introduction: In equestrian sports where horses exercise at submaximal levels, the identification of some clinical conditions leading to poor performance can remain difficult. The aim of this study was to design a graded exercise test, applicable in the field and to test the hypothesis that measured heart rate (HR) and lactate (LA) differ between healthy and subclinically-affected horses, according to diagnosis.

Methods: Fifty-four sport horses regularly participating in competitions were studied. A total of 106 examinations were performed, 85 were poor performance investigations (PP) and 21 were controls (C) in healthy performing individuals. The exercise test consisted of 4 consecutive 2-minute steps, 2 at trot and 2 at canter. Heart rate (HR), ECG and lactate (LA) were recorded. Blood samples, tracheal wash and bronchoalveolar lavage were systematically collected. Other examinations were performed when indicated. Differences in HR and LA between groups were compared using an ANOVA. Repeatability was checked in 12 horses.

Results: Tests were highly reproducible. Diagnoses included upper (15%) and lower (48%) respiratory, cardiac (12%), locomotor (25%), and muscular (17%) disorders. Although horses did not systematically exceed the lactate threshold, HR and LA at steps 2, 3 and 4 and recovery HR differed significantly between groups ($P<0.001$–0.03). The rate of increase in LA and HR varied according to diagnosis, with LA reaching significantly higher values in horses affected with cardio-respiratory conditions.

Conclusions: Field exercise testing in sport horses during the competitive season is a simple procedure that allows an objective measurement of the horse's capacity to exercise. The analysis of HR and LA provides indications for specific diagnostic examinations and helps assess the functional impairment caused by subclinical disease.

Ethical Animal Research: This study was based on clinical cases referred to the Equine Sports Medicine Practice. Horse owners referred their horses and consented to all procedures described. **Sources of funding:** none declared. **Competing interests:** none.

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FIELD INVESTIGATION OF POOR PERFORMANCE IN THOROUGHBRED RACEHORSES

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Introduction: Racehorses suffering from subclinical diseases are frequently kept in training until they fail expectations during competition. The diagnosis of medical causes of poor performance remains challenging in the field. The aim of this study was to estimate the prevalence of subclinical causes of poor performance in working Thoroughbreds (TB) and to determine which clinical field tests could most appropriately identify them.

Methods: During the course of a training session, a complete clinical screening was performed in 11 healthy controls (C) and 38 poor performing (PP) TB without obvious clinical signs. Exercising ECG and GPS monitoring, blood sample analysis including oxidative stress markers, Doppler echocardiography, respiratory function testing (RFT), post-exercise endoscopy, tracheal wash (TW) and broncho-alveolar lavage (BAL) were performed. Statistics included ANOVA and Spearman's test ($P<0.05$).

Results: Subclinical causes of PP included muscular, respiratory and cardiac disorders. Variables derived from velocity and HR showed significant differences between groups. Lower airway diseases (LAD) were the most frequently diagnosed (33/38), causing significant differences in RFT. Endoscopic observations correlated with TW cytology, but unlike BAL analysis, were insufficient to diagnose all LAD cases. Supra- or ventricular premature beats were present only in the PP group but were not associated with echocardiographic abnormalities. Glutathione peroxidase activity was significantly lower in PP.

Conclusions: Velocity, ECG and HR monitoring during a training session can be used as non-specific predictive indicators of performance, help assess functional consequences of diseases and guide ancillary diagnostic investigations. As LAD is highly prevalent, BAL sampling would be indicated in any case of poor performance.

Ethical Animal Research: The study was approved by the Ethical committee of the University of Liege. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** University of Liege, Frank Duncombe Laboratory and the French Haras Nationaux. **Competing interests:** none.

ROLE OF THE CO_2 SYSTEM IN REGULATING INTRAMUSCULAR HYDROGEN ION CONCENTRATION DURING EXERCISE IN HORSES

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Introduction: Elimination of CO_2 from skeletal muscle (mCO_2) during exercise is important for the regulation of intramuscular $[H^+]$ ($[mH^+]$). Carbonic anhydrase (CA) catalyses the hydration/dehydration reaction of CO_2 to facilitate its elimination. We hypothesised that extracellular CA inhibition and subsequent increase in PCO_2 will result in increased $m[H^+]$ and decreased exercise performance.

Methods: Six horses were exercised on a treadmill until fatigue without (Con) and with acetazolamide (CA inhibitor) treatment (Acz; 30 mg/kg, 30 min before exercise). Arterial and mixed-venous blood was sampled, and biopsies collected from gluteal muscles at fatigue. Whole blood (wb) arteriovenous difference across the muscle (a-v) of $[Na^+]$, $[K^+]$, $[Cl^-]$, $[Lac^-]$, [SID], and PCO_2, mCO_2 and $m[H^+]$ were determined. Paired t-test was used to assess statistical significance ($P<0.05$).

Results: Duration of exercise to fatigue was not affected by Acz. Compared to Con, Acz increased mCO_2 (Con 16.5(mean) ± 3.6(SE), Acz 20.5 ± 2 mmol/L) and mixed-venous and arterial PCO_2 but had no effect on VCO_2. No difference was observed in $m[H^+]$ between Con (118 ± 18 nmol/L) and Acz (99 ± 16 nmol/L). Acetazolamide did not affect plasma $[H^+]$. Based on the a-v differences Acz increased $[Na^+]$wb and $[K^+]$wb release, increased $[Cl^-]$wb uptake and did not affect $[Lac^-]$wb release by the muscle. Acz decreased a-v[SID]wb (Con 6.2 ± 2.8 mmol/L, Acz –0.3 ± 2 mmol/L).

Conclusions: Although PCO_2 and mCO_2 were elevated no difference in $[mH^+]$ was observed. An increase in $m[H^+]$ due to elevated mCO_2 was attenuated by strong ion fluxes between intramuscular and vascular spaces. Strong ion exchanges are important in attenuating $m[H^+]$ and maintaining exercise performance.

Ethical Animal Research: The study protocols were approved by the Animal Care Committee of the University of Guelph according to the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, Ottawa, Ontario). **Sources of funding:** Ontario Ministry of Agriculture and Food - Equine Program, Department of Clinical Studies at the University of Guelph, Canadian Institutes of Health Research and the Slovenian Research Agency grant P4-0053. **Competing interests:** none.

GENETIC COMPONENT OF ENDURANCE ABILITY

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Introduction: Arabian horses have the best records in long distance endurance racing (80–160 km). The endurance horses are interesting models to calculate the heritability (h^2) of exercise ability. Heritability indicates the percentage of trait transmitted by parents to their descendants (h^2 = Genetic variance/Total variance). In human athletes, heritability of physiological traits calculated using twin design were 0.12, 0.72 and 0.32 for VO_{2max}, Heart Rate max and endurance capacity respectively. Heritabilities of 0.28 and 0.06 for speed and placing respectively are reported in endurance horses. The aim of this study was to calculate the heritability of recovery heart rate, cardiac recovery time and average speed in long distance endurance racing.

Methods: The results of 132 endurance races (80–160 km) from 2007 to 2011 were analyzed. The database included 4421 horses of known pedigrees representing 655 sires with a mean of 18.5 products per sire. The data were analyzed using Restricted Estimator of Maximal Likelihood (REML, VCE6) method with animal mixed model including country, ride distance, age, gender, common environment as fixed effects and animal genetic as random effect.

Results: Heritabilities (SE) were 0.12 (0.06), 0.15 (0.02) and 0.46 (0.08) for speed, recovery heart rate and cardiac recovery time respectively.

Conclusions: The speed and heart rate recovery seem more influenced by training and environmental effects than the cardiac recovery time which is more influenced by genetics. This high heritability is consistent with heritability of V200 found in French Trotter population previously and may indicate a putative quantitative trait linkage.

Ethical Animal Research: Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** National Studs (IFCE) and the Arabian Horse Association (ACA). **Competing interests:** none.

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BIOCHEMISTRY, HAEMATOLOGY, ENDOCRINOLOGY AND THERMOREGULATION IN EXERCISE

INTRA- AND INTER-DAY VARIABILITY IN PLASMA BICARBONATE CONCENTRATION IN SEDENTARY HORSES

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Introduction: Alkalinizing agents have been given to horses before exercise to provide exogenous buffering effects. The objective of this experiment was to determine the intra- and inter-day variability of resting plasma tCO_2. The hypothesis was that tCO_2 would vary measurably during a multi-day quarantine.

Methods: Eight acclimated sedentary horses had jugular venous samples collected into 10-ml heparinized evacuated glass tubes 3 times daily (7 AM, 11 AM, and 3 PM) for 5 consecutive days. Horses ate 5.3 kg alfalfa hay at both 7 AM and 3 PM after blood sampling. Plasma tCO_2 was determined within 1–3 hours of sampling using an automated chemistry analyzer. Mean tCO_2 for all 8 horses was calculated for each of the 15 sampling intervals. Results were compared by Repeated Measures ANOVA. When there was no apparent day-to-day effect, data for all horses were pooled by time-of-day (n = 40 samples for each of 3 times-of-day) and compared by the Student's t test to determine time-of-day effect. $P<0.05$ was considered significant.

Results: Mean tCO_2 over the 15 sampling intervals ranged from 28.9-to-31.6 mmol/L and was lower at 11 AM (Day 1) and 3 PM (Days 1, 3, and 4) compared to 7 AM on Day 1 ($P<0.05$). Individual plasma tCO_2 ranged over 4-to-7 units (low 26, high 34, widest range 26–32, narrowest 28–31). Mean pooled tCO_2 differed by time-of-day, with 11 AM (30.1 ± 0.2, P = 0.011) and 3 PM (29.8 ± 0.2, $P<0.001$) values lower than 7 AM values (31.0 ± 0.2).

Conclusions: Plasma tCO_2 differed significantly by time-of-day with considerable intra- and inter-individual variability.

Ethical Animal Research: The study was approved by the University of Illinois Institutional Animal Care and Use Committee. **Sources of funding:** Fédération Equestre Internationale and Maria Caleel Fund for Equine Sports Medicine Research. Support for Dr. Bergstrom was provided by the Merial Veterinary Scholars Program. **Competing interests:** none.

ACID-BASE ALTERATIONS IN BARREL RACING QUARTER HORSES OF DIFFERENT FITNESS LEVELS ASSESSED VIA THE SIMPLIFIED STRONG ION APPROACH

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Introduction: The evaluation of acid-base balance of horses is important because it detects alterations that affect their performance and health. There are studies relating these changes with endurance type effort, but few regarding sprint exercise. The purpose of this study was to determine whether the fitness level of Quarter Horses performing barrel racing could be differentiated by quantitative acid-base analysis.

Methods: For this purpose, 10 horses were distributed into two groups: high (HF) and lower (LF) fitness as defined by the trainer. GPS and frequency meter were used for the measurements. Venous blood gas analysis was performed using a portable analyzer (I-stat) before and after exercise. Strong ion difference (SID), anion gap (AG), strong ion gap (SIG) and concentration of non-volatile buffers (Atot) were determined. Variables before and after exercise were compared within and between groups by t-test ($P<0.05$).

Results: The average track distance was 125 m. There were no differences between maximum or average velocity and heart rate, which were 30.76 ± 0.53 km/h, 22.85 ± 0.28 km/h, 206 bpm ± 3.91 versus 27.7 ± 2.4 km/h, 21.94 ± 0.3 km/h and 203 bpm ± 5.77 for the HF and LF groups, respectively. SID values before exercise were significantly lower for the LF group. Both groups showed a significant increase in Atot and AG, while pH, HCO_3^-, SIG and SID decreased significantly after exercise.

Conclusions: Metabolic acidosis was observed using both the traditional and the quantitative approach. It was not possible to differentiate the fitness level of horses in the study.

Ethical Animal Research: All procedures were approved by the institutional animal care and use committee of the university (CEUA, Process: 008481/11). Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** UNESP Reitoria (PIBIC). **Competing interests:** none.

POST-RACE PLASMA ANGIOTENSIN CONVERTING ENZYME IN THOROUGHBRED HORSES WITH OR WITHOUT FUROSEMIDE

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Introduction: Exercise-Induced Pulmonary Haemorrhage (EIPH) results from hypertensive episodes associated with intense exercise and can negatively impact athletic performance. Angiotensin converting enzyme (ACE) secretion from pulmonary endothelium is increased during pulmonary disease. This study investigated the effects of furosemide administration on ACE activity, and hypothesised a positive association between EIPH and post-race plasma ACE.

Methods: Blood samples from 73 horses were collected after racing. ACE activity was measured using a fluorometric method. Respiratory endoscopy determined occurrence and grade of EIPH. Comparison of horses treated or not with furosemide before racing and the occurrence of EIPH were conducted by independent sample t-tests. In addition, regression analysis was used to investigate the association between ACE activity and EIPH grade for all horses. Other variables analysed included time to endoscopy and haematocrit.

Results: Mean ACE activity in horses not treated with furosemide (n = 47; 91.7 ± 15.9) was significantly higher (P = 0.002) than in treated horses (n = 26; 79.6 ± 13.9). When EIPH positive horses were analysed separately, those treated with furosemide (n = 20; ACE 78.2 ± 11.9) had significantly lower ACE (P = 0.009) than non-treated horses (n = 17; ACE 90.9 ± 15.9). Regression analysis indicated a polynomial relationship between mean ACE activity and EIPH grade (0, 1, 2 or 3 + 4 + 5; adjusted r2 = 0.99; $P<0.001$). No other comparisons were statistically significant.

Conclusions: Results indicated associations between ACE activity, EIPH grade and furosemide administration and investigation of the potential use of ACE as a biomarker for EIPH severity in controlled studies are warranted.

Ethical Animal Research: This research was conducted with approval from The Animal Ethics Committee at WINTEC, and informed owner consent was obtained for horses included in this study. **Sources of funding:** partially funded by a Research Grant from WINTEC. **Competing interests:** none.

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LACTATE TRANSPORT IN RED BLOOD CELLS BY MONOCARBOXYLATE TRANSPORTER MCT1 AND ITS ACCESSORY PROTEIN CD147 IN BRAZILIAN SPORT HORSES OF DIFFERENT PERFORMANCE LEVELS

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Introduction: The Brazilian sport horse (BH) was specifically developed for jumping competition. Monocarboxylate transporters are key regulators of cell pH and therefore facilitate co-transport of protons and lactate. Lactate transport in red blood cells (RBCs) is bimodal (high [HT] and low transport [LT]) and is related to CD147 levels. This study aimed to quantify MCT1 and CD147 protein levels in BH after an incremental jumping test (IJT).

Methods: Sixteen horses were distributed into two groups: low-level jumpers (LL, n = 8), able to jump up to 1.00 m, and high-level jumpers (HL, n = 8), able to jump up to 1.20 m. The IJT consisted of three steps, each with forty jumps at increasing heights (0.40, 0.60, and 0.80 m). Blood samples were collected before and after the IJT. The YSI 2300 method was used to determine plasma lactate concentration [Lac]p. RBC membranes were purified, and MCT1 and CD147 levels were measured by Western blotting. A Student's t-test and Pearson's correlation ($P<0.05$) were employed.

Results: The HL group displayed lower [Lac]p, indicating prevalence of the aerobic pathway in this group. The results showed no difference in the expression of the transporters between times or groups and a positive correlation ($r = 0.67$, $P<0.0001$) between MCT1 and CD147. In both groups [Lac]p didn't correlate with the amount of MCT1 or CD147. Most of the horses appeared to have high lactate transport activity. The CD147/MCT1 ratios for LL and HL were equal.

Conclusions: BH showed no difference in MCT1 and CD147 expression between conditioning levels.

Ethical Animal Research: All procedures were approved by the institutional animal care and use committee of the university (CEUA, Process: 019281/13). Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo (Process 2011/11080-0). **Competing interests:** none.

ACID-BASE IMBALANCES IN ENDURANCE RACING USING TRADITIONAL AND QUANTITATIVE APPROACHES

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Introduction: Acid-base disturbances are frequently assessed using traditional variables PCO_2, pH, and bicarbonate. This study aimed to compare the changes in acid-base balance in horses performing a CEI*** endurance race (160 km) using traditional and quantitative methodologies.

Methods: Blood samples were drawn before racing, at the vet-gate (66 km), immediately after racing, and 2 and 15 h after racing from 18 participating horses. The mean velocity of the horses was 17 km/h. PCO_2 and pH were measured (I-Stat). Strong ion difference (SID), anion gap (AG), strong ion gap (SIG) and total volatile buffers (Atot) were calculated using formulas from the literature. Data for finishers (F) and non-finishers (N) was compared with an unpaired test. Data collected for finishers at all time-points was compared by repeated ANOVA followed by Tukey's test.

Results: PCO_2 was lower in group N at the 66 km time-point. In group F, AG had a bimodal behaviour, declining immediately after exercise and then increasing at 2 and 15 hours. There was a slight increase in pH post-exercise. After 2 hours, Atot increased while PCO_2 decreased. Compared to SIG immediately after racing, SIG at 2 and 15 hours after racing was decreased. Traditional methodology suggested mild respiratory and metabolic alkalosis, possibly due to hyperventilation of the finishers. Quantitative methodology suggested metabolic acidosis reflected by decreasing SID trend and a significant increase of Atot.

Conclusions: Acid-base imbalances are present in endurance racing horses and differences were found between the traditional and quantitative methods of assessment.

Ethical Animal Research: All procedures were approved by the institutional animal care and use committee of the university (CEUA, Process: 007347/13). Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo (Process 2013/00449-8). **Competing interests:** none.

"POSITIVE" PLASMA TCO_2 TESTS IN RACEHORSES IN NEW YORK, MAINE, AND CALIFORNIA RESULT FROM DAY-TO-DAY INSTRUMENT VARIABILITY

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Introduction: Many jurisdictions measure plasma tCO_2 to determine if racehorses may have been administered exogenous alkalinizers illegally ("milkshakes"). Our hypothesis was that plasma tCO_2 test results vary within a racing jurisdiction due to inter-day instrument variability.

Methods: Individual or daily mean plasma tCO_2 test results from New York (n = 584) and Maine (n = 443) Standardbreds and California Thoroughbreds (n = 23,686) were obtained through legal discovery in adjudicated cases where trainers were accused of tampering with horses resulting in tCO_2 concentrations ≥37.0 mmol/L. Daily results were grouped by days on which no horse tested ≥37.0 mmol/L (LOW) or days on which at least one horse tested ≥37.0 mmol/L (HIGH). HIGH days were further subsampled by eliminating "positive" test results ≥37.0 mmol/L (MODIFIED HIGH). With $P<0.05$ considered significant, differences between groups were compared by Student's t test for means and Mann-Whitney Rank Sum Test for medians.

Results: HIGH tCO_2 means (32.9 ± 2.6, 35.2 ± 3.4, 32.6 ± 2.7) and medians (32.9, 35.4, 32.6) for all 3 jurisdictions were higher than LOW means (31.7 ± 2.3, 31.9 ± 4.3, 31.5 ± 0.7) and medians (31.8, 32.9, 31.5) ($P<0.001$). Even when "positive" tests ≥37.0 were eliminated, MODIFIED HIGH means (32.5 ± 2.2, 33.6 ± 2.6, 32.4 ± 2.5) and medians (32.7, 34.2, 32.5) were still higher than LOW ($P<0.001$).

Conclusions: Unless the population tCO_2 mean varies significantly daily, it must be concluded that instrument variability resulted in day-to-day differences in plasma tCO_2 test results. Without appropriate quality control including correction of individual suspect results to a contemporaneous standard curve, horse trainers may be accused unfairly of tampering when day-to-day instrument variability contributes to positive plasma tCO_2 test results.

Ethical Animal Research: This was a retrospective study investigating laboratory results obtained through adjudication of legal cases before various racing commissions. **Sources of funding:** none. **Competing interests:** none.

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HEART RATE RESPONSES IN SHOW-JUMPERS OVER A THREE-DAY TRAINING SESSION

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Introduction: The objective of this study was to provide more information regarding the relationship between heart rate (HR) and performance in show-jumpers.

Methods: Ten mixed breed horses (9.1 ± 1.9 yrs) were assessed at a British Equestrian Federation (BEF) World Class Performance three-day training session. Riders received coaching during warm-up (WU) and after jumping a 15 fence (1.35–1.45 m) course once on day-1, twice on days 2 & 3; on day-3 the second round was an 8-fence jump-off. Clinical assessments were performed, and plasma lactate concentrations determined, pre- and post-exercise. Take-off distance (TDF) was determined using high-speed motion capture. The coach graded horse jumping technique (validated methodology). HR was recorded telemetrically from WU until 10 mins post-exercise. Variables were compared using regression procedures/Spearman correlation and paired Students t-test ($P<0.05$).

Results: HR was not significantly different between days or consecutive jumping rounds. Mean HR was lower in WU than during the course (127 ± 11 vs 136 ± 16: $P = 0.027$), likely related to increased speed during the course, although HRpeak tended to be higher during WU day- 1 possibly reflecting excitement. Mean HR increased for each quarter during the course. Higher mean course and third-quarter HR were associated with higher blood lactate concentrations ($P = 0.047$), greater gluteal and longissimus dorsi muscle spasm on palpation ($P<0.01$), more faults ($P = 0.044$) and lower technique scores ($P = 0.019$). TDF was negatively correlated with mean course and third-quarter HR, indicating that closer take-off may be harder work ($P<0.01$).

Conclusions: HR increases throughout a jumping course. Higher HRs may be associated with jumping inefficiency and muscle spasm, with potential performance implications for multi-day competitions.

Ethical Animal Research: Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** The World Class Development Programme is funded by the Department for Culture, Media and Sport, through Exchequer and National Lottery funds, via UK Sport. **Competing interests:** none.

SERUM IMMUNOGLOBULIN CONCENTRATIONS IN RACING ENDURANCE HORSES

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Introduction: Serum immunoglobulin fractions were evaluated in horses participating in multi-day endurance exercise.

Methods: Serum IgGa, IgGb, IgG(T), IgA, and IgM were measured using horse-specific ELISA kits in 44 trained horses prior to racing, after each day of racing 50 miles per day and in 15 untrained matched control horses. Data were analyzed via repeated measures ANOVA ($P<0.05$).

Results: 44, 18 and 9 horses completed one, two and three days of racing, respectively. Serum IgA before racing (1.97 ± 0.14 mg/dL) was similar to controls (1.48 ± 0.19) and after one, two or three days of racing (1.98 ± 0.13, 1.80 ± 0.15 and 1.76 ± 0.26). Serum IgM before racing (1.02 ± 0.05 mg/dL) was similar to controls (1.08 ± 0.12) and after one, two or three days of racing (1.03 ± 0.06, 0.91 ± 0.08 and 0.89 ± 0.11). Serum IgG(T) before racing (6.10 ± 0.41 mg/dL) was similar to controls (6.29 ± 0.58) and after racing one, two or three days (6.23 ± 0.45, 6.62 ± 0.63, and 6.65 ± 1.34). Serum IgGa was 2.41 ± 0.10 mg/dL before racing, and was similar to controls (2.45 ± 0.22) and each day of racing (2.58 ± 0.14, 2.39 ± 0.20, and 2.61 ± 0.22, on days one, two and three of racing). Resting serum IgGb concentration was significantly lower in control (5.71 ± 0.54 mg/dL) than trained horses (7.65 ± 0.41).

Conclusions: Serum IgGb is higher in trained endurance horses, perhaps reflecting immune stimulation related to disease exposure or more frequent vaccination.

Ethical Animal Research: All procedures were approved by the Oregon State University Institutional Animal Care and Use Committee. Owner informed consent was obtained for all horses in the study. **Sources of funding:** Department of Biomedical Sciences at Oregon State University. **Competing interests:** none.

PRELIMINARY RESULTS ON CD147 EXPRESSION IN RED BLOOD CELLS OF ARABIAN HORSES

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Introduction: During anaerobic glycolysis for energy production, lactate and H^+ ions accumulate in muscle fibers and pass into the bloodstream. Monocarboxylate transporter type 1 and its ancillary protein CD147 transport H^+ and lactate ions from the plasma into red blood cells, thereby maintaining acid/base homeostasis and retarding systemic acidosis and fatigue. The aim of this study was to compare the levels of CD147 protein in the red blood cells of Arabian horses with different levels of athletic ability.

Methods: Blood samples were taken from 12 Arabian horses before exercise. The horses were divided into two groups: untrained horses (UH, n = 6) and 160-km endurance horses (FEI***, n = 6). The amount of CD147 in the plasma membranes of their red blood cells was determined by Western blotting analysis using a human-specific anti-CD147 antibody previously validated for horses. The medians were compared by Mann-Whitney Rank Sum test at 5%.

Results: CD147 protein with a molecular mass of approximately 45 kDa was found in the red blood cell membranes of the studied horses. The CD147 expression levels of the two groups were not different ($P = 0.065$). The observed median values (min–max) were 0.50 (0.37–1.26) and 0.27 (0.09–0.69) arbitrary optical density units for the FEI*** and UH group respectively.

Conclusions: Studies must be performed with more horses to determine if a different level of CD147 expression exists in FEI*** horses related to their intensive training programs or to the genetic traits of each horse.

Ethical Animal Research: All procedures were approved by the institutional animal care and use committee of the university (CEUA, Process: 019314/13). Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo. Process: 2012/24193-0. **Competing interests:** none.

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EFFECT OF A 90-KM ENDURANCE RACE ON RESISTANCE TO OXIDATION AND INFLAMMATION LEVELS IN 6-YEAR OLD HORSES

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Introduction: The aim of this study was to determine whether serum anti-oxidant defenses are impaired by endurance exercise and to investigate possible correlation with performance in the race.

Methods: Blood samples were obtained from 23 Arabian or half-Arabian horses (6 years old) the day before (T0) and 30 minutes after (T1) a 90-km race. Performance records available included speed, heart rate 10 and 30 minutes after arrival and the rating by veterinarians based on recovery and gait (5 categories from 'eliminated' to 'excellent'). Absorbance at 413 nm (A413), at 650 nm (A650) and absorbance of Rose Bengal (RB) were measured in sera at T0 and T1 (a higher absorbance means a lower antioxidant capacity). Relationships between blood variables and between T0 values and race results were evaluated using correlation analysis and means comparisons.

Results: All horses finished the race with an average speed of 16.3 km/h. Three horses were eliminated for lameness, one was excellent and the others were distributed between the other three categories. After the race, a significant increase of RB, A413, A413-A650, PT and CK ($P<0.001$) was observed. At T0, RB and CK were significantly correlated ($r = 0.52$, $P<0.01$). At T1, absorbance values were significantly correlated with muscle enzymes and haptoglobin ($r = 0.50–0.67$, $P<0.05$). The speed of the horse was negatively correlated with RB at T0 ($r = –0.42$, $P<0.05$). No significant difference in absorbance values could be detected between the recovery categories.

Conclusions: A higher resistance to oxidative stress was observed before the race in the best performing horses.

Ethical Animal Research: The study design was approved by the Ethics Committee for the Alfort Veterinary School and the University of Paris-Est under number 12/07/11-1; owner informed consent was obtained before any manipulation on horses. **Sources of funding:** National Studs (IFCE), the Eperon Funds and the Arabian Horse Association (ACA). **Competing interests:** none.

BLOOD BIOCHEMISTRY AND HEMOGASOMETRY OF HORSES PERFORMING INCREMENTAL JUMPING AND VELOCITY TESTS

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Introduction: Biochemical and hemogasometric parameters have been used to evaluate equine performance. This study evaluated blood biochemical and hemogasometry variables of horses during incremental jumping and speed tests.

Methods: Eighteen horses performed a jumping test with four steps over a vertical obstacle of 1.10 m height (10, 15, 20 and 25 jumps, respectively) totaling 70 jumps, and a speed test with six steps of one minute each with increasing speed from 2 to 8 m/s. Blood samples were collected before and immediately after each test to determine plasma lactate, hematocrit, total plasma protein, glucose, uric acid and hemogasometry variables. Results were analysed by ANOVA and compared by 5% Tukey test.

Results: Immediately after tests the hematocrit and total plasma protein decreased from 52.4% to 38.9%, and from 7.2 to 6.7 g/dl respectively, and the glucose increased from 98.3 to 109.8 mg/dl ($P<0.05$). The lactate and uric acid concentrations were higher with speed testing (7.97 ± 4.8 mmol/l and 1.33 ± 0.8 mg/dl respectively) than jumping testing (3.92 ± 3.0 mmol/l and 1.24 ± 0.9 mg/dl respectively). The blood pH was lower after speed testing (7.36 ± 0.1) than the jumping test (7.40 ± 0.04) ($P<0.05$). Blood pCO_2, Na and K concentrations increased more after the speed test (53.38 ± 7.3 mmHg/l; 139 ± 3.0 mEq/l; 4.7 ± 0.8 mEq/l respectively) than after the jumping test (49.34 ± 3.2 mmHg/l; 138.3 ± 2.3 mEq/l; 4.51 ± 0.6 mEq/l respectively) ($P<0.05$). Blood pO_2 was not different among tests ($P>0.05$).

Conclusions: Incremental jumping and velocity tests are useful tools for assessing performance of jumping horses. Differences among tests could evidence a smaller anaerobic contribution during jumping test.

Ethical Animal Research: The study was approved by UFRRJ Ethics Committee on Animal Research: number 263/2012. **Sources of funding:** CNPq, CAPES and FAPERJ. **Competing interests:** none.

A STANDARDISED GAITED FIELD TEST PRODUCES CHANGES IN BLOOD BIOMARKERS AND HEART RATE IN FOUR-BEAT GAITED HORSES

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Introduction: There is little information about biochemical and physiologic responses to exercise in Brazilian gaited horses. The aim of this study was to evaluate blood biomarkers and heart rate (HR) in gaited horses during a standardized gaited field test (SGT).

Methods: Sixteen horses performed a SGT (10 min warm-up, 30 min of marcha gait and 15 min recovery). Blood samples and HR were collected at rest, after warm-up, after 15 (F + 15) and 30 min (F + 30) of marcha, and after recovery. Assays were performed to measure glucose, lactate, total plasma protein, hematocrit, urea, creatinine, glutamine, glutamate, triglycerides, total cholesterol, Ca, P, Mg, Na, Cl and K. HR was measured using a HR monitor. Results were analyzed using one-way or two-way ANOVA. Post-hoc analyses included the Holm-Sidak test, Student's t-tests, and Pearson's correlation analyses. The null hypothesis was rejected when $P<0.05$.

Results: [Glucose] and [lactate] increased during SGT ($P<0.05$). A significant interaction between phase of SGT and fitness level was observed for [glucose] but not for [lactate]. The SGT also produced increases ($P<0.05$) in hematocrit, [TPP], [urea], [creatinine], [triglycerides], [P] and [K] and a reduction ($P<0.05$) in [Ca]. HR increased from ~47 bpm at rest, to ~140 bpm at F + 15 and F + 30 ($P<0.05$).

Conclusions: This study documents submaximal aerobic exercise, as performed during the GST is sufficient to alter energy biomarkers in gaited horses.

Ethical Animal Research: The study was approved by UFRPE's animal research committee (#62/2007-CTA/DZ/UFRPE). **Sources of funding:** CAPES, IRCA Animal Nutrition and Cascatinha, Recanto da Serra, Annette and Abreu horse farms. **Competing interests:** none.

REAL-TIME MONITORING OF THE CORE BODY TEMPERATURE OF ENDURANCE HORSES DURING FIELD EXERCISE

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Introduction: Continuous monitoring of core body temperature may provide useful information about the thermal response to metabolic heat

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load and environmental challenges. The aim of this study was to evaluate a telemetric gastrointestinal (GI) pill (Equivital®) measuring gastrointestinal temperature (GT) during endurance exercise. It was hypothesised that a temperature and metabolic heat profile could be established.

Methods: The GI pill was administered to 6 horses by nasogastric tube (n = 3) or modified-drench gun (n = 3), the day before competition. During competition, GT, skin temperature and heart rate (HR) were recorded (by Equivital® belt). Other data collected included body weight and rectal temperature (RT), GPS/HR monitor information, and environmental conditions using a data logger. Metabolic heat production was calculated [1] and GT correlations were explored using simple correlation estimates ($P<0.05$).

Results: Data were collected from 6 horses during 80 km (n = 4 horses) and 100 km (n = 2) competition days with mean data loss of 7.4% (±2.4%). GT profiles showed increasing temperature with cumulative metabolic heat production, and time to reach maximum GT was 2.14–6.29 h (maximum GT range: 38.4–39.5°C). During exercise (7 h 6 min ± 1 h 3 min duration), mean and maximum speeds ranged from 10.1–14.5 km/h and 23.5–30.8 km/h, respectively. No correlation was found between GT and HR, skin, or environment temperature. Maximum GT increased with bodyweight ($r = 0.775$, $P = 0.08$). GT was systematically higher than RT (mean difference +0.46°C).

Conclusions: GT was recorded successfully during competition with minimal data loss. The GI pill has great potential as a method to further investigate thermoregulation in horses under field conditions.

Ethical Animal Research: All studies were performed under the Adelaide Animal Ethics Committee, University of Adelaide. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** The equipment for the project was funded by the Faculty of Science 'Grant for minor equipment', University of Adelaide, Australia. **Competing interests:** none.

Reference

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ELECTROLYTE SUPPLEMENTATION PRIOR TO PROLONGED SUBMAXIMAL EXERCISE RESULTS IN DECREASED PCO_2 AND TCO_2 DURING EXERCISE AND RECOVERY

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Introduction: Electrolyte supplementation prior to exercise is known to benefit both performance and recovery; however its effects on acid-base status remain not well characterized. The purpose of this study was to use the physicochemical approach to provide a comprehensive time-course of the acid-base alterations that occur following pre-exercise electrolyte supplementation in horses. It was hypothesized that electrolyte supplementation will attenuate the alkalosis occurring with prolonged moderate intensity exercise.

Methods: In a randomized crossover experiment four conditioned mares were given, via nasogastric tube, 3 L of a commercial electrolyte solution (ES; designed to fully replace sweat electrolyte and fluid losses), or water alone (Control), 1 hour before they exercised on a treadmill at 30% VO_{2max} until voluntary fatigue (inability to keep pace with belt speed). Blood was sampled from jugular venous catheters at frequent intervals before, during, and until 20 h of exercise recovery, and analyzed for all dependent and independent plasma acid-base variables. Differences ($P<0.05$) over time and between groups were assessed by 1- or 2-way repeated measures ANOVA.

Results: Horses exercised for 57.5 ± 10.3 and 72.5 ± 17.0 min and lost 2.73 ± 0.82 and 4.63 ± 1.35 L in Control and ES trials, respectively. ES horses had lower pCO_2 and total carbon dioxide (TCO_2) concentrations during exercise and early recovery. Control horses exhibited decreased $[H^+]$. pCO_2 was the primary contributor to changes in $[H^+]$ and $[TCO_2]$, while there were no differences in strong ion difference ([SID]) or total weak acid concentration ([Atot]).

Conclusions: Electrolyte supplementation prior to prolonged exercise leads to sustained effects on equine acid-base variables, even in the absence of significant changes in plasma electrolytes or hydration.

Ethical Animal Research: The experiments and use of animals were approved by the University of Guelph Animal Care Committee, in accordance with the guidelines of the Canadian Council on Animal Care. **Sources of funding:** Ontario Horse Racing Industry Association and the Natural Sciences and Engineering Research Council of Canada. **Competing interests:** none.

CARDIOVASCULAR AND RESPIRATORY

ACCURACY OF A POINT-OF-CARE LACTATE ANALYZER FOR MEASURING BLOOD AND PLASMA L-LACTATE CONCENTRATIONS IN EXERCISING STANDARDBREDS

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Introduction: The Accutrend® Lactate (formerly Accusport®) is a low cost point-of-care L-lactate analyser. The analyser is widely used in the training of horses and has been used for research studies and to assist the clinical management of critically ill horses. The analyser is reported to be linear from 0.8–22.0 mmol/L (blood) and 0.7–26.0 mmol/L (plasma); however, test performance at high L-lactate concentrations in equine blood and plasma has not been well documented. The purpose of this study was to determine the accuracy of the Accutrend® Lactate analyzer over a wide range of L-lactate concentrations in paired blood and plasma samples.

Methods: Duplicate jugular blood samples were collected from 8 trained Standardbreds before, during, and after a simulated race on a treadmill into 4 mL partially evacuated tubes containing lithium heparin. Blood and plasma L-lactate concentrations were measured using a colorimetric lactate-oxidase mediated reaction (BM-Lactate® test strips and Accutrend® Lactate) and the reference enzymatic method (L-lactic dehydrogenase spectrophotometry at 340 nm using samples deproteinized with 8% perchloric acid). Data was analysed using Deming regression and Bland-Altman plots adjusted for repeated measures ($P<0.05$).

Results: The reference method measured a wide range of L-lactate concentrations in 128 blood (0.2–23.8 mmol/L) and 127 plasma (0.3–41.1 mmol/L) samples. The Accutrend® Lactate analyzer provided an accurate method of L-lactate measurement, but only when blood and plasma L-lactate concentrations were <12.5 mmol/L (79% of samples) and <15.0 mmol/L (64% of samples), respectively.

Conclusions: Blood L-lactate concentrations >12.5 mmol/L and plasma L-lactate concentrations >15.0 mmol/L measured by the Accutrend® Lactate analyzer underestimate the true value by a variable amount when compared with the enzymatic reference method.

Ethical Animal Research: The study was approved by the Institutional Animal Care and Use Committee at Purdue University. **Sources of funding:** Supported, in part, by a grant from the Indiana Horse Racing Commission. **Competing interests:** none.

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EXPERIMENTAL DETERMINATION OF THE APPARENT VALUE FOR THE FIRST DISSOCIATION CONSTANT (pK_1') OF THE PLASMA CARBONIC ACID – BICARBONATE BUFFER SYSTEM IN 115 TRAINED STANDARDBREDS

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Introduction: Calculating the total plasma concentration of carbon dioxide ($ctCO_2$) from the results of blood gas and pH analysis assumes that the value for pK_1' is constant and approximates 6.095. The objective of this study was to verify both assumptions in equine plasma.

Methods: Duplicate jugular blood samples were collected pre-race from 115 trained Standardbreds into 3 mL partially evacuated plastic tubes containing lithium heparin. Blood pH (pHb) and partial pressure of CO_2 (pCO_2) were measured in one tube using a blood gas and pH analyser (Radiometer ABL-700). The second tube was anaerobically centrifuged and $ctCO_2$ was measured (Beckman EL-ISE analyser). Plasma pK_1' was calculated for each horse using the Henderson-Hasselbalch equation, measured values for pHb, pCO_2, and plasma $ctCO_2$, and the value for the solubility of CO_2 in plasma at 37C (S = 0.0307 {mmol/L}/mmHg) whereby: $pK_1' = (pHb + 0.01) - \log10(\{ctCO_2 - S \times pCO_2\}/\{S \times pCO_2\})$, with the term (pHb + 0.01) representing the pH in plasma. The calculated value for pK_1' was standardized to pHb = 7.40 using the Severinghaus equation such that $dpK_1'/dpH = -0.044$. Non-parametric statistical procedures were used to determine the median value and 95% confidence interval (CI) for pK_1'.

Results: The median value for pK_1' at pHb = 7.40 was 6.097, and the 95% CI for pK_1' was 6.063–6.123.

Conclusions: The 95% CI for pK_1' in equine plasma from trained Standardbreds is narrow and includes the assumed fixed value of 6.095. Our findings support the use of blood gas and pH analysis to calculate plasma $ctCO_2$ in order to detect the pre-race administration of alkalinizing agents in horses.

Ethical Animal Research: The study was approved by the Institutional Animal Care and Use Committee at Purdue University. **Sources of funding:** Supported, in part, by a grant from the Indiana Horse Racing Commission. **Competing interests:** none.

DIFFERENTIATING PATHOLOGICAL FROM PHYSIOLOGICAL LEFT VENTRICULAR HYPERTROPHY BY QUANTIFYING MYOCARDIAL FUNCTION

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Introduction: Differentiating left ventricular (LV) dilatation caused by aortic regurgitation (AR) from athletic eccentric LV hypertrophy can be challenging. In human medicine, tissue Doppler imaging (TDI) and 2D speckle tracking (2DST) are used to distinguish 'athlete's heart' from pathological conditions.

Methods: 40 healthy horses (group 1) were compared to 13 horses with moderate AR (group 2) and 11 horses with severe AR (group 3), all with an end diastolic LV internal diameter (LVIDd) within maximal reference limits (≤14.2 cm). Fractional shortening (FS) was measured from a short-axis M-mode at chordal level. Peak TDI velocities during systole (S), early (E) and late (A) diastole were measured in the LV free wall (LVFW) and interventricular septum (IVS). Global longitudinal strain was measured by 2DST. Groups were compared by a one-way ANOVA with Bonferroni correction.

Results: The study included Warmbloods (n = 52), trotters (n = 9) and Thoroughbreds (n = 3), used for jumping, dressage, racing and eventing. Group 2 (15 ± 5 years; P<0.001) and 3 (20 ± 4 years; P<0.001) were older than group 1 (8 ± 4 years). LVIDd in group 3 (13.1 ± 0.7 cm) was higher compared to group 1 (11.5 ± 0.8 cm; P<0.001) and 2 (12.0 ± 0.8 cm; P = 0.003) but FS was not different. Peak S in IVS was higher in group 2 (6.6 ± 1.5 cm/s; P = 0.001) and 3 (9.0 ± 1.7 cm/s; P<0.001) compared to group 1 (4.4 ± 1.8 cm/s), indicating hyperkinetic septal motion. Peak E in LVFW was lower in group 2 (18.6 ± 5.2 cm/s;P = 0.001) and 3 (19.3 ± 3.9 cm/s; P = 0.006) compared to group 1 (24.4 ± 4.6 cm/s), indicating decreased early filling velocity. Longitudinal strain was not different between groups.

Conclusions: TDI shows potential for differentiating AR-induced LV remodeling from athletic eccentric hypertrophy in horses.

Ethical Animal Research: Horses with aortic regurgitation were examined in a prospective clinical study with owner informed consent. **Sources of funding:** The corresponding author is a postdoctoral research fellow of the Research Foundation Flanders. **Competing interests:** none.

LONGITUDINAL FOLLOW-UP OF BRONCHOALVEOLAR LAVAGE CYTOLOGY IN STANDARDBRED RACEHORSES

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Introduction: Bronchoalveolar lavage (BAL) fluid cytology is a major diagnostic method for detecting exercise-induced pulmonary haemorrhage (EIPH) and inflammatory airway disease (IAD) in racehorses. Our hypothesis was that training and racing would increase the incidence of IAD and EIPH.

Methods: On three different occasions (day-1, –30 and –60), BAL fluid was obtained from both lungs of 14 Standardbred trotters with similar age and training level. Cytological inclusion criteria were neutrophils >10%, and/or mast cells ≥5%, and/or eosinophils ≥5% for IAD, and haemosiderophage/macrophage (H/M) ratio >20% for EIPH. Since previously found not to be equivalent, both samples from each horse were independently investigated. Horse status ('IAD','EIPH', 'mixed', or 'control') at each time point was based on cytological profiles from both lungs. Cell proportions were compared by repeated measures ANOVA.

Results: Haemosiderophage proportions and H/M were significantly higher on day-60 compared to day-1. No significant difference was observed for other cell proportions. On day-1, 5 horses were 'control', 2 'IAD', 2 'EIPH, and 5 were 'mixed'. On day-30, 3 horses were 'control', 4 'IAD', 2 'EIPH', and 5 were 'mixed'. On day-60, 3 horses were 'control', 0 'IAD', 3 'EIPH', and 8 'mixed'. Despite the different horses' distribution within each category, an overall increase in EIPH was observed. Five of the 7 horses classified 'control' or 'IAD' on day-1 presented EIPH on day-30 and/or –60. EIPH was detected in 11/14 horses (78.6%) on day-60, versus 7/14 horses (50%) on day-1.

Conclusions: The incidence of EIPH, but not IAD, significantly increased during a racing season. This evolution should be considered when assessing BAL fluid cytology in racehorses.

Ethical Animal Research: The study was approved by the regional Animal Ethics Committee (n°CEEA.2012.179) and all owners signed a consent form. **Sources of funding:** IFCE (National Studs) and SECF (French Trotters Racing Association). **Competing interests:** none.

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THE PREVALENCE OF ABNORMAL BREATHING PATTERNS DURING EXERCISE AND ASSOCIATIONS WITH DYNAMIC UPPER RESPIRATORY TRACT OBSTRUCTIONS

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Introduction: During canter and gallop most horses maintain a locomotor-respiratory coupling (LRC) ratio of one breath every stride. Occasional big respiratory cycles (BRC) are described when a single breath is taken over two strides. This is considered a transient but normal feature of ventilation. However, a change to a more persistent 2:1 LRC ratio is considered to be 'abnormal'. It has been suggested that upper respiratory tract (URT) disorders could result in a horse adopting an abnormal breathing pattern, although current information is limited.

Methods: Simultaneous audio and URT endoscopy recordings from 365 horses referred for treadmill evaluation were analysed. Breathing pattern at canter and gallop was categorised at each one minute speed step:

1. Normal: 1:1 with <2 BRC
2. Normal: 1:1 with 2 BRC
3. Abnormal: breathing pattern disrupted by repetitive and frequent BRC's
4. Abnormal: alternating between periods of 2:1 and 1:1
5. Abnormal: 2:1 throughout

Data analysis was performed using PASW 18.0. A chi-square test was used to identify associations between breathing pattern and presence of URT obstructions.

Results: During the entire exercise test 30% of horses had an abnormal breathing pattern at canter or gallop, of which 23% had a 2:1 breathing pattern. The prevalence of abnormal breathing patterns was higher at low canter speeds than during strenuous exercise. There was a significant association between breathing pattern during strenuous exercise and the presence of an URT obstruction ($P = 0.008$).

Conclusions: Loss of a 1:1 LRC ratio during canter and gallop is more common than previously realised and may be associated with dynamic URT obstructions.

Ethical Animal Research: Retrospective analysis of case records. **Sources of funding:** none declared. **Competing interests:** none.

BIG RESPIRATORY CYCLES DURING TREADMILL EXERCISE

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Introduction: Big respiratory cycles (BRCs) are a sporadic but normal occurrence during galloping exercise. Their relationship to performance capacity as reflected by maximal oxygen consumption (VO_{2max}) is unclear.

Methods: 7 Thoroughbreds completed multiple incremental exercise tests up to 13 m/s on a treadmill to determine their VO_{2max} while wearing a mask that measured inspired and expired volumes (VI, VE) and VO_2 on a breath-by-breath basis. Frequency and duration of BRCs were related to speed and VO_{2max} using regression analysis. Numbers of BRCs for each test were counted and the duration, VI, VE and VO_2 of each BRC determined and compared to the corresponding average values of the 5 breaths following the BRCs, and VO_{2max} for the exercise bout. Significance was set at $P<0.05$.

Results: The frequency of BRCs was 64% when all speeds and runs were considered. The number and appearance of BRCs varied considerably between horses. BRC numbers were greatest at 6 and 13 m/s and least at 9 m/s, and significantly related by polynomial regression ($r2 = 0.90$). BRC duration was not related to breathing frequency when all BRCs were considered due to the existence of two distinct clusters of BRCs. The duration of those lasting <1.25 s was inversely related to speed ($r2 = 0.77$) as were those >1.25 s in length ($r2 = 0.55$). BRC VI and VE were greater than normal VI and VE and the difference increased linearly ($r2 = 0.89$ and 0.87, respectively) as speed increased from 6 m/s (VI: $8.0 \pm 0.6\%$; VE: $8.9 \pm 0.7\%$ greater) to 13 m/s (VI: $42 \pm 2\%$; VE: $57 \pm 3\%$ greater). Individual VO_{2max} was not related to the number, length or volume of BRCs.

Conclusions: The frequency, duration and size of BRCs do not affect VO_{2max} and vary considerably within a given treadmill test and between horses.

Ethical Animal Research: Procedures were approved by the Institutional Animal Care and Use Committee (protocol #3807). **Sources of funding:** Funding was provided by the home department. **Competing interests:** none.

EFFECT OF HEAD AND NECK POSITION ON UPPER AIRWAY FUNCTION IN STANDARDBRED RACEHORSES

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Introduction: Poll flexion may play an important role in the development of dynamic upper respiratory tract (URT) collapse. However, limited investigations have been performed in Standardbred racehorses.

Methods: Eight Standardbreds were examined during training, once flexed and once in extension, using a randomised crossover design. Head position was maintained using modified check-reins and head-neck angle was recorded. On each occasion horses performed 2 rounds of exercise of 2400 m at ~35 km/h. Speed and heart rate (HR) were measured and blood obtained before and after exercise. URT endoscopy was performed during the second round. Videos were blinded and URT function assessed. HR, plasma lactate (Lac) and speed were compared for different head positions using paired t-tests. Cortisol was analysed using repeated-measures ANOVA and URT function using Fisher's exact tests and paired t-tests ($P<0.05$).

Results: Data were excluded for one horse where head-neck angle could not be measured. Flexed and extended head-neck angles differed significantly (mean: 85.0° vs 97.8°; $P = 0.001$). All horses showed some form of URT collapse during exercise, although this was only deemed clinically significant in 4 horses with a history of abnormal noise. These horses showed additional abnormalities during flexion. However, mean severity scores did not reach statistical significance ($P = 0.08$). No significant difference was found for speed, HR or Lac. A significant increase in cortisol was found with exercise ($P<0.001$). The interaction between time and head position approached significance ($P = 0.09$) with higher concentrations of cortisol generally occurring in extension rather than flexion.

Conclusions: URT abnormalities may be exacerbated with poll flexion. The use of equipment to modify head position may induce a stress response.

Ethical Animal Research: This study was approved by the University of Adelaide Animal Ethics Committee and owner informed consent obtained. **Sources of funding:** University of Adelaide honours projects. **Competing interests:** none.

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EVALUATION OF A PORTABLE SYSTEM DESIGNED TO MEASURE VENTILATORY PARAMETERS AND OXYGEN CONSUMPTION IN UNRIDDEN HORSES

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Introduction: Measurement of maximal oxygen consumption (VO_{2max}) is the gold standard for assessing aerobic capacity, but in horses requires use of stationary equipment and a high-speed treadmill. Study objectives were to evaluate a portable system designed to measure VO_2 in a field setting and to describe these measurements in a cohort of untrained Thoroughbreds.

Methods: Ventilatory parameters and VO_2 were measured in 41 untrained Thoroughbreds (16 colts, 25 fillies, 19.8 ± 1.4 months) using an ergospirometry and oximetry mask with customized software validated for horses galloping on a treadmill with an open-circuit flow-through gas collection system. Horses were instrumented with a GPS-HR monitor, light-weight mask and data-capture equipment. Data was collected as horses travelled riderless up a 2% inclined woodchip track. Pre- and 5 minute post-exercise plasma lactate ([La5]), HRpeak, maximal velocity (Vmax) and exercise distance were recorded and analysed by unpaired t-test and Pearson's correlation ($P \leq 0.05$).

Results: All horses tolerated the mask and saddle pad. Exercise distance was 1700.5 ± 33.4 m, Vmax 15.0 ± 1.4 m/s, HRpeak 221.2 ± 9.6 bpm and [La5] 22.7 ± 5.5 mmol/l. Average VO_2peak, based on 6 breaths, was 117.7 ± 24.1 ml/kg/min, breathing frequency 125.8 ± 7.1 bpm, inspiratory volume 13.2 ± 1.7 L, expiratory volume 13.4 ± 1.9 L and minute ventilation 1669.3 ± 193.3 L/min. VO_2peak was not significantly different between colts (112.2 ± 27.7 ml/kg/min) and fillies (121.4 ± 21.3 ml/kg/min). There were no correlations between VO_2peak and other measured parameters. There was a positive linear correlation between VO_2peak and HRpeak ($P = 0.046$, $R2 = 0.16$).

Conclusions: VO_2peak values were similar to published reports for yearling Thoroughbreds. The high exercise intensity and linear relationship between VO_2peak and HRpeak provides confidence in the system for field studies of aerobic capacity in untrained, unridden horses.

Ethical Animal Research: Institutional Animal Research Ethics Committee approval was obtained. **Sources of funding:** Science Foundation Ireland (11-P1- 1166). **Competing interests:** None.

COMPARISON OF BRONCHOALVEOLAR LAVAGE AND TRACHEAL ASPIRATE RESULTS IN 64 NON-THOROUGHBRED HORSES PRESENTING WITH RESPIRATORY SIGNS OR EXERCISE INTOLERANCE

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Introduction: It has been debated which airway sampling method, bronchoalveolar lavage (BAL) or tracheal aspirate (TA), is superior in evaluating equine respiratory disease. Most studies suggest BAL is the best sampling method due to better correlation with lung histopathology. In this study we compared BAL and TA results of athletic non-Thoroughbred horses with chronic respiratory signs or exercise intolerance during 2009–2013.

Methods: 64 horses, 2–25 years of age (16 Coldbloods, 30 Warmbloods, 11 Standardbreds, 7 ponies) with recurrent or chronic (duration >1 month) respiratory signs or exercise intolerance that were subject to both BAL and TA sampling at rest were included. Neutrophil counts >5% in BAL and >20% in TA were considered abnormal. Statistical analysis was performed with Spearman's correlation coefficient.

Results: Both BAL and TA were abnormal in 29 horses and normal in 26 horses. 7 horses had only abnormal TA and 2 horses only abnormal BAL. Correlation was found between the amount of mucus in the trachea and both BAL ($P = 0.002$) and TA ($P<0.001$) neutrophil percentage. Neutrophil ($P<0.001$), eosinophil ($P<0.001$), lymphocyte ($P = 0.008$) and macrophage ($P = 0.004$) percentages correlated between BAL and TA.

Conclusions: In our university hospital population, mucus in the trachea was a good indicator of airway neutrophilia. Unlike previous studies, in only 14% of the horses the interpretation of BAL and TA result was different with TA being slightly more sensitive at detecting neutrophilia. However, a gold standard for detecting inflammation in the equine airways is still lacking and the type and severity of the disease may greatly influence the best choice of sampling method.

Ethical Animal Research: Retrospective analysis of case records. **Sources of funding:** none. **Competing interests:** none.

CHUCKWAGON FIELD STUDY: HIGH-SENSITIVITY CARDIAC TROPONIN-T INCREASES WITH EXERCISE BUT IS NOT CORRELATED WITH THE PRESENCE OF ARRHYTHMIAS DURING EXERCISE

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Introduction: In chuckwagon racing, dubbed the "half-mile of hell", teams often race multiple consecutive days. Horses are in peak condition, however rare cases of sudden death attributed to cardiovascular failure occur. There are concerns regarding cumulative racing and its impact on the risk of sudden death. The objective was to determine if cumulative exercise affects integrity of the myocardium, assessed by high-sensitivity troponin-T (cTnT), and if the presence of arrhythmias during race or recovery affects plasma cTnT concentrations.

Methods: Heart rate and electrical activity were recorded on 148 horses (9 ± 2.7 years) during racing days 1 and 2. 750 plasma samples were analysed (374 pre-exercise, 376 post-exercise) comparing cTnT before and after activity (paired t-test). Associations between consecutive racing days, age and cTnT were analysed (ANOVA, Pearson Correlation). The relationship between arrhythmias and cTnT was also examined.

Results: cTnT increased significantly with exercise (pre: 4.52 ng/L ± 0.17, post: 5.20 ng/L ± 0.23, $P<0.001$). However cTnt was not affected by cumulative exercise. A correlation between age and cTnT was found. 4235 arrhythmic events were recorded. No association existed between cTnT (post-exercise) and the presence of arrhythmias during exercise and recovery.

Conclusions: This is the first study examining the effect of exercise on high-sensitivity cTnT in horses and first large-scale study on chuckwagon horses. Racing increases cTnT, however values remain low, suggesting myocardial damage of little clinical significance. Chuckwagon horses have long careers therefore age and cTnT could be further examined. cTnT was not affected by the presence of arrhythmias during exercise, indicating chuckwagon racing is well tolerated.

Ethical Animal Research: The study was approved by the Veterinary Sciences Animal Care Committee of the University of Calgary (AC120049) and onwer informed consent was obtained. **Sources of funding:** Clinical Research Fund from the University of Calgary's Faculty of Veterinary Medicine. Summer Studentships were funded by NSERC. **Competing interests:** none.

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CHUCKWAGON FIELD STUDY ON 148 HORSES: RACING CONSECUTIVE DAYS AFFECTS BASELINE BLOOD ELECTROLYTES, pH AND LACTATE BUT DOES NOT INCREASE THE PREVALENCE OF CARDIAC ARRHYTHMIAS

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Introduction: Chuckwagon racing involves 4 Thoroughbred horses pulling a 600 kg wagon through a figure-8 pattern and running a 1000 m dash. Horses can race four days in a row and there are concerns regarding the contribution of cumulative racing to electrolyte imbalances, arrhythmias, and sudden death, and its impact on recovery. The objective was to investigate the effects of cumulative racing on plasma electrolytes, lactate and prevalence of cardiac arrhythmias.

Methods: 148 horses (9.0 ± 2.7 years) from 13 teams were monitored for heart rate and ECG activity on racing days 1 and 2. Differences in plasma electrolytes, pH and lactate (pre-vs. post-exercise and between days) were analysed (paired t-test). Associations between electrolytes and the presence of arrhythmias during racing and recovery (Pearson correlation) were examined.

Results: 377 ECGs (82 incomplete due to lost electrode) and 750 plasma samples (374 pre, 376 post) were analysed. 4235 arrhythmic events (21.6% warm-up, 37.6% racing, 20.4% recovery) were recorded and were not different between days 1 and 2. They mainly comprised supraventricular premature contractions during warm-up and racing (48% and 81.4% respectively) and ventricular premature contractions during recovery (29.2%). A significant association was found between post-exercise electrolytes and the presence of arrhythmias during racing ($P<0.001$, $r = 0.17$). Consecutive racing significantly increased post-exercise lactate on day 2 ($P<0.001$). Pre-exercise pH and electrolytes were significantly different on day 2.

Conclusions: An association exists between post-exercise electrolytes and occurrence of arrhythmias during consecutive racing, however it is of little clinical significance. Ventricular arrhythmias are as prevalent in chuckwagon horses as in other disciplines. Overall, repetitive racing is well tolerated.

Ethical Animal Research: The study was approved by the Veterinary Sciences Animal Care Committee of the University of Calgary (AC120049) and owner informed consent was obtained. **Sources of funding:** Clinical Research Fund from the University of Calgary's Faculty of Veterinary Medicine. Summer Studentships were funded by NSERC. **Competing interests:** none.

HEMODYNAMIC AND ENDOCRINE CHANGES ASSOCIATED WITH SPLENIC RESERVE MOBILIZATION

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Introduction: In theory, splenic reserve mobilization should elicit a cardiopulmonary baroreceptor mediated neuroendocrine response (the Gauer-Henry reflex).

Six intact (IN) and 5 (SP) splenectomized Standardbred mares (421–491 kg, ~9 yr) performed 10 min of treadmill exercise (80% VO_{2max}) to test the hypothesis that hemodynamic and endocrine responses to exercise would differ between IN and SP horses

Methods: Blood samples were obtained before and at 1, 2, 4, 6, 8 and 10 min of exertion. Right ventricular pressure (RVP), right atrial pressure (RAP), arterial pressure (MAP), heart rate (HR), and cardiac output (CO) were measured and total peripheral resistance (TPR) calculated. Plasma atrial natriuretic peptide (ANP) and vasopressin (AVP) concentrations were measured using RIA. Data analysis used ANOVA for repeated measures and SNK. Significance was set at $P<0.05$.

There were no differences ($P>0.05$) between IN and SP for any variable at rest. All variables except TPR and RAP increased ($P<0.05$) during exertion in both IN and SP. Means (±SE) for RAP (18 ± 3 vs. 1 ± 2 mmHg), CO (255 ± 21 vs.177 ± 19 L/min), MAP (147 ± 3 vs. 116 ± 8 mmHg), and ANP (66 ± 3 vs. 50 ± 8 pg/mL) were greater for IN vs. SP horses at 10 min of exertion. Mean plasma AVP (95 ± 19 vs. 23 ± 3 pg/mL) was greater ($P<0.05$) for SP than for IN horses at 10 min of exertion.

Ethical Animal Research: The study was approved by the Institutional Animal Care and Use Committee. **Sources of funding:** Ohio Thoroughbred and Standardbred Research Fund and the New Jersey State Initiative on Equine. **Competing interests:** none.

EFFECTS OF AEROBIC TRAINING ON SYSTOLIC, DIASTOLIC AND MEAN BLOOD PRESSURE IN ARABIAN HORSES

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Introduction: Exercise causes several physiological changes resulting from autonomic and hemodynamic adaptations, but there are few reports about the behaviour of blood pressure (BP) during physical activity in horses, as well as the effect of training on BP.

Methods: 9 untrained Arabian horses underwent two treadmill evaluations (before and after 90 days of aerobic training at 60% HRmax) at 6% incline with incremental speed increases every 3 minutes (commencing at 2.5 m/s and reaching 12.5 m/s). The facial artery was cannulated with a 20 G catheter and systolic blood pressure (SBP), mean (MAP) and diastolic (DBP) blood pressures were recorded with a multiparameter monitor (GE Dash 3000 model). Data were evaluated by two-way ANOVA for repeated measures and Bonferroni multiple comparison ($P<0.05$).

Results: During initial evaluation SBP increased in a gradual and uniform manner as speed increased, reaching a maximum of 208 mmHg at a speed of 10 m/s. After training, SBP behaved similarly during treadmill evaluation, however values were lower until 10 m/s ($P<0.001$), and the maximum value of SBP obtained was 218 mmHg at 12.5 m/s. DPB was not significantly different ($P = 0.124$). MAP emulated the behaviour of SBP ($P = 0.012$).

Conclusions: A decrease in SBP and MAP during identical treadmill evaluations before and after 90 days of aerobic exercise (60% HR max) indicated that training decreased cardiac effort required for the maintenance of physical activity.

Ethical Animal Research: procedures were approved by the University of São Paulo. **Sources of funding:** none declared. **Competing interests:** none.

ARRHYTHMIAS IN THOROUGHBREDS DURING AND AFTER TREADMILL AND RACETRACK EXERCISE

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Introduction: Healthy and poorly performing horses can display arrhythmias during exercise and immediately afterwards. The relationship

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of arrhythmias to exercise intensity or type, and their frequency of recurrence, are poorly understood. Clarification of these issues was the objective of this investigation.

Methods: Electrocardiograms were recorded on 9 Thoroughbreds during maximal and submaximal exercise on a racetrack (RA) and treadmill (TM). The frequency of atrial and ventricular arrhythmias for RA and TM were compared using Fisher's Exact Test.

Results: 65 workouts (TM = 46, RA = 19; median[range]: 4/horse[2–14]) were performed. Exercising arrhythmias were detected in 4/9 horses (12/65 workouts) and there were post-exercise arrhythmias in 7/9 horses (20/65 workouts). A single exercising electrocardiogram per horse often did not display all arrhythmias detected over several workouts. 3/9 horses exhibited arrhythmias during the first and second runs while the other 6 had none. 4/9 had 1 post-exercise arrhythmia. Of 6 horses with >3 runs, 1 displayed arrhythmias each time, 3 never had arrhythmias and 2 sometimes showed arrhythmias during exercise (1/9 and 4/8 workouts, respectively). All 6 intermittently had post-exercise arrhythmias (median[range]: 50% [9–75%] of workouts). Presence of arrhythmias was positively related to intensity and all occurred at ≥94%HRmax. The frequency of arrhythmias following RA (29%) and TM (12.5%) was not statistically different.

Conclusions: Gallops on TM or RA were equally effective for detecting arrhythmias. The presence/absence of exercising arrhythmias was more repeatable than post-exercise arrhythmias. Arrhythmias are more likely to be detected at maximal or near-maximal intensities. A larger population needs to be studied before firm conclusions are drawn.

Ethical Animal Research: The study was approved by an Institutional Animal Care and Use Committee protocol. **Sources of funding:** Department of VCS, Washington State University. **Competing interests:** none.

CARDIOPULMONARY FUNCTION IN THOROUGHBRED HORSES RUNNING UPHILL AND DOWNHILL ON AN INCLINED TREADMILL

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Introduction: Eccentric muscle contractions ("negative" work, e.g., downhill running) affect human skeletal muscle differently than concentric contractions, increasing muscle size and strength more than concentric exercise training with reduced oxygen consumption (VO_2). Better understanding of skeletal muscle and cardiopulmonary responses to downhill training may indicate a role for this in Thoroughbred training. We hypothesised that Thoroughbreds would reduce metabolic energy cost and cardiopulmonary function when they ran at equivalent speed on a declined treadmill compared with running horizontally or uphill.

Methods: Five well-trained Thoroughbreds ran for 2-min incremental exercise intervals on a treadmill at 1.7, 3.5, 6.0, 8.0 and 10.0 m/s on a +4% incline, 0% incline (horizontal) and –4% decline in random order on different days. Cardiopulmonary and O_2-transport variables were measured and analysed with two-way repeated-measures ANOVA and Holm-Šidák pairwise-comparisons.

Results: Horses ran at all inclines with identical stride frequency and length. At identical uphill speeds they had significantly higher (vs. horizontal) mass-specific VO_2 (43% mean increase) and CO_2 production (VCO_2/Mb), cardiac output (Q/Mb), heart rate, arterial CO_2 tension ($PaCO_2$), and reduced arterial O_2 tension and saturation; tidal volume tended to be higher ($P = 0.060$). Downhill running reduced (vs. horizontal) VO_2/Mb (19% mean decrease), VCO_2/Mb, Q/Mb and $PaCO_2$. Fractional energy cost increase (incline) and reduction (decline) decreased linearly with increasing speed.

Conclusions: Thoroughbreds run downhill with lower energy cost than on the level or uphill and the cost changes with speed. It remains to be determined if eccentric training induces skeletal muscle changes in horses.

Ethical Animal Research: The study was approved by the Animal Welfare and Ethics Committee of the Equine Research Institute, Japan Racing Association. **Sources of funding:** none. **Competing interests:** none.

VALIDATION OF A NOVEL HIGH-SENSITIVITY CARDIAC TROPONIN-T ASSAY IN HORSES

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Introduction: Cardiac troponin-I (cTn-I) has been previously validated in horses. Newer high sensitivity troponin assays have recently become available that are now the standard in human cardiology. The objective was to validate the high-sensitivity cardiac troponin-T (hscTn-T) assay for use in horses and to determine the biological variation within and between horse breeds.

Methods: Samples were analysed using the hscTn-T assay reagent (Roche,Cobas c501). Method validation included linearity, lower limit of quantitation (LLQ), freeze-thaw stability, within-and between-run precision, and comparison with cTn-I (Abbott iSTAT, Deming regression). Normal range and biological variation included healthy horses (n = 120; age = 7.2 ± 0.5 years (SE)) representing 5 breeds.

Results: Assay was linear from 3–391 ng/L. The LLQ was validated at 3 ng/L. Stability of samples was unaffected by 3 freeze-thaw cycles. Within-run mean (±SD) was L1 = 6.50 (±0.97), L2 = 10.1 (±0.88), L3 = 15.3 (±0.82) ng/L and between-run mean (±SD) was L1 = 12.2 (±1.03), L2 = 57.0 (±4.82), L3 = 256 (±23.1) ng/L. Comparison with cTn-I assay showed excellent correlation (range: 8–3535 ng/L, r = 0.9998). Bias was evident in the regression results [hscTnT = (0.6153 ± 0.002809)*(cTnI*1000) – (11.53 ± 5.462)]. The 95th and 99th percentile of the normal distribution in healthy horses was 4 and 6 ng/L. Between breed, diurnal effect and between day variation was not detectable due to low concentrations of hscTnT.

Conclusions: This study established reference intervals for hscTn-T assay in healthy horses and the performance characteristics of hsTn-T assay in horse samples. Normal population distribution was mostly below the detection limit of the assay. The hscTn-T assay will likely establish the standard in early detection of cardiac pathologies in horses.

Ethical Animal Research: This study was approved by the Veterinary Sciences Animal Care Committee (protocol number AC12-0049). **Sources of funding:** UCVM internship fund. **Competing interests:** none.

COST OF TRANSPORT AS A DISCRIMINATOR OF CONDITIONING IN HORSES SUBMITTED TO INCREMENTAL JUMPING TESTS

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Introduction: Heart rate (HR) and blood lactate (LAC) are biomarkers whose responses to exercise are related to physical fitness. This study evaluated the cost of transport (CT) by assessing the cardiovascular and metabolic efficiency of jumping horses using an incremental jumping test.

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Methods: Sixteen Brazilian Sport horses of two fitness levels were used: high-level jumpers (HL, n = 8) and low-level jumpers (LL, n = 8). Tests were performed in a sandy jumping arena at a mean velocity of 18 km/h; the tests were distributed into three steps of 4 laps of 74 m, each with 10 obstacles. Each stage consisted of simple obstacles that were 0.40, 0.60 and 0.80 m high. The costs of transport were determined as (HRstep-HRrest/speed) and (LACstep-LACrest/speed). During intervals between each stage, blood samples were drawn to determine LAC (mmol/L). Student's t-test was used for the unpaired samples (P<0.05).

Results: There was no difference in the stage 0.40 m for HR. There were differences in the HL and LL groups, respectively, in the HR related to the effort in the obstacle series 0.60 m [1.7 ± 0.2 bpm/(km/h) and 2.0 ± 0.2 bpm/(km/h) (P = 0.02)]; 0.80 m [1.5 ± 0.2 bpm/(km/h) and 1.7 ± 0.2 bpm/(km/h); (P = 0.04)], and in the LAC for the 0.40 m obstacle [0.05 ± 0.04 mmol/L/(km/h) and 0.10 ± 0.03 mmol/L/(km/h); P = 0.04]; 0.60 m [0.07 ± 0.06 mmol/L/(km/h) and 0.14 ± 0.03 mmol/L/(km/h); (P = 0.006)] and 0.80 m [0.11 ± 0.04 mmol/L/(km/h) and 0.19 ± 0.05 mmol/L/(km/h); P = 0.009].

Conclusions: The CT differentiated horses of high and low level jumping fitness. The HR method (rather than LAC) is particularly useful because it is simple, inexpensive and accessible to trainers and veterinarians.

Ethical Animal Research: All procedures were approved by the institutional animal care and use committee of the university (CEUA, Process: 019281/13). **Sources of funding:** FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo (Process 2011/11080-0). **Competing interests:** none.

EVOLUTION OF ECHOCARDIOGRAPHIC DIMENSIONS WITH AGE AND TRAINING IN YOUNG ENDURANCE HORSES

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Introduction: Increasing cardiac dimension with endurance training is well known in adult athletes. However, adaptation of the heart to training in growing Arabian type horses is less well documented.

Methods: During the national finals for young endurance horses, standardized echocardiographic examinations were performed before the races by two experienced examiners. In 2011 left ventricle (LV) was assessed in 74 horses aged 4-years (18), 5-years (33) and 6-years (23). In 2012 left atrial (LA) dimensions and the great heart vessels were measured in 122 horses aged 4-years (32), 5-years (40) and 6-years (50). Data were normally distributed; age groups and gender were compared by one-way ANOVA (Holm-Sidak test for multiple comparisons, significance P<0.05); relationships between echocardiographic dimensions and body weight (BWT) were assessed by linear regressions.

Results: BWT was highest in 5-year old horses. LV dimensions increased with age, but for most measurements the difference was significant only for the group of 6-year old horses. The stroke volume (SV) increased (P = 0.0016) and the heart rate decreased (P = 0.06) with increasing age, leading to a constant cardiac output in all age groups. There was a weak relationship between BWT and SV. Two dimensional LA measurements increased between 4 and 5 years but remained constant between 5 and 6 years. LA area measurements and LA functional indices showed no evolution between the groups. Aortic dimension increased with age but pulmonary artery dimension showed no such increase. No influence of gender on echocardiographic dimensions and function could be found.

Conclusions: Great heart vessels, LA and LV dimensions showed different growth patterns between 4 and 6 years of age.

Ethical Animal Research: Procedures were approved by the Ethics Committee for the Alfort Veterinary School and the University of Paris-Est under number 12/07/11-1; owner informed consent was obtained. **Sources of funding:** National Studs (IFCE), The Eperon Funds and the Arabian Horse Association (ACA). **Competing interests:** none.

CARDIAC MORPHOLOGY AND HEART MURMURS IN ELITE EVENTING AND DRESSAGE HORSES

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Introduction: Training related cardiac adaptation is documented in Standardbreds and Thoroughbreds, including an increased incidence of AV valve regurgitation. In Warmbloods less is known about training effects on cardiac function or subclinical valve regurgitation.

Methods: Fifteen adult untrained Warmbloods (aged 3.8 ± 0.7 years) were compared with fifteen adult Warmblood eventers (aged 11.4 ± 0.9 years) competing at CIC2* (group 1, n = 5) and CCI2*, CIC3*, CCI3* and CCI4*levels (group 2, n = 9) and fifteen healthy adult Warmbloods competing at advanced dressage (group 3, n = 5), Prix Saint Georges, Intermediate or Grand Prix (group 4, n = 10) levels (mean age 10.3 ± 0.7 years). The presence of heart murmurs, valve regurgitation and echocardiographic parameters were evaluated, and training intensity was scored. A correlation was found between bodyweight and not height. Differences in cardiac morphology were compared using General Linear Models with post-hoc Bonferroni correction (SPSS version 20), using group and bodyweight as covariates. Significance was set at P<0.05.

Prevalence of audible heart murmurs and valvular regurgitation was higher in eventers (0–20% and 29–79%, respectively and dressage horses (7–40% and 7–60%, respectively) than controls (0–20% and 7–20%, respectively). Interventricular septum in diastole (IVSd, left ventricular mass (LVM) and LV mean wall thickness (LVMWT) were greater in groups 2 and 4 than controls; group 4 also had a greater pulmonary artery diameter (PAD) and LV posterior wall (LVPWd). Group 1 showed a greater LVMWT; group 4 a greater end point septal separation (EPSS). Training intensity influenced in both categories IVSd and MWT; in eventers also the right ventricular diameter in diastole and in dressage horses also LVPWd, LVM, EPSS and PAD.

Elite eventers and dressage horses showed an increased prevalence of audible murmurs and valvular regurgitation. Cardiac adaptations resembled those associated with resistance and endurance training. Increased performance level and training intensity accompanied increased cardiac muscle thickness and volume. These effects should be considered during sales and performance evaluations.

Ethical Animal Research: Evaluated by the Animal Ethics Committee of Utrecht University and owner consent was obtained. **Sources of funding:** Faculty of Veterinary Medicine, Utrecht University. **Competing interests:** none.

DESCRIPTION OF A FIELD EXERCISE TEST IN CROSS-BRED QUARTER HORSES IN VENEZUELA: PRELIMINARY V200 AND V170 VALUES

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Introduction: Field exercise tests (FET) can assist design of individualized training programs. Although FETs have been used in other breeds, there are limited reports for Quarter horses (QH) and cross-bred QH which are widely used in traditional Venezuelan equestrian activities. The aim of this study was to describe the implementation of an FET in cross-bred Quarter horses in Venezuela.

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Methods: Six horses (3 stallions and 3 mares) performed a FET consisting of a four-step incremental increase in speed (91 ± 10; 136 ± 3; 271 ± 20; 439 ± 43 m/min). Each cycle lasted 3 to 5 min with 3 minutes of rest between each. Environmental temperature was 32°C. Heart rate and speed were recorded (Polar Equine RS800CX®) and the speed to reach a heart rate of 200 and 170 beats/min was calculated by linear regression. The rate of heart rate decline was determined 5 minutes after the last step. Linear regression analysis, descriptive statistics and an unpaired t- test (Graph Pad Prism 5.0) were utilized to identify gender differences ($P<0.05$).

Results: Gender did not influence the results. V200 and V170 were slightly higher in stallions (617 ± 98 and 465 ± 66 m/min, respectively) than in mares (511 ± 60 and 387 ± 60 m/min, respectively). The rate of heart rate decline was slightly more rapid in mares (25 ± 7 beats/min) compared to stallions (13 ± 5 beats/min).

Conclusions: Although a low number of horses were evaluated, these preliminary data suggest that gender has limited influence on fitness of cross-bred Quarter horses used in local Venezuelan equestrian activities under tropical environmental conditions. The mean V200 and V170 values obtained were lower than those reported for Thoroughbreds and Standardbreds, but similar to those reported in young racing Quarter horses.

Ethical Animal Research: The study was approved by the Faculty of Veterinary Science, Central University of Venezuela Ethical Research Committee. **Sources of funding:** "San Antonio Farm" owned by Perez family and CDCH-UCV (PG-11-8095-2011). **Competing interests:** none.

MUSCLE AND BONE

DETERMINATION OF MYOFIBER TYPES OF MANGALARGA MARES AND THEIR HYBRIDS

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Introduction: There are no published studies that characterize the different myofiber types of Mangalarga (ML) horses and hybrids. This study aimed to characterize and compare muscle fibers of the gluteus medius of ML mares and young and inactive mules resulting from the breeding of these mares with a Brazilian donkey.

Methods: Percutaneous biopsies were performed in 11 mares and their foals (10 mules), at a depth of 60 mm. Fiber types were determined by comparing identical images using both the staining techniques of myofibrillar adenosine triphosphatase (mATPase) and nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR). Stains for mATPase were pre-incubated in acid medium followed by alkaline incubation. The frequency (F%) and relative total area (RTA%) of fiber types I, IIA and IIX were analysed. The cross-sectional area (CSA, μm2) was quantified by the NADH-TR technique. The means were compared using ANOVA followed by Tukey test ($P<0.05$).

Results: The frequency of type IIA fibers was greater than types I and IIX. Type IIX fibers had higher CSA than I and IIA. The RTA of type IIX fibers was greater than type IIA fibers followed by type I. The higher RTA value of IIX fibers resulted from higher CSA. There was no difference between the variables for mares compared to the mules.

Conclusions: ML mares and mules that resulted from breeding with the Brazilian donkey have a high prevalence of type IIA fibers in the gluteus medius. This fatigue-resistant fiber uses both metabolic pathways, glycolytic and oxidative, to obtain energy during muscle contraction, thus giving the ML mares and mules good performance in horseback riding, marching contests and endurance rides.

Ethical Animal Research: All procedures were approved by the institutional animal care and use committee of the university (CEUA, Process: 01370/12). **Sources of funding:** Fundação de Amparo a Pesquisa do Estado de São Paulo. Process: 04655-9/2012. **Competing interests:** none.

THE EFFECT OF LAND AND UNDERWATER TREADMILL TRAINING ON METABOLIC RESPONSES TO EXERCISE AND PROPERTIES OF SUPERFICIAL DIGITAL FLEXOR AND GLUTEAL MUSCLE

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Introduction: Resistance training provided by underwater (UWT) treadmills may enhance strength and fatigue resistance of superficial digital flexor muscle thereby reducing tendon injury. This study examined effects of UWT and land (LT) treadmill training on SDF and gluteal muscle.

Methods: Six unfit horses (3 x 2 crossover design, 60d detraining period) walked on UWT and LT for 5d/week for 8 weeks (up to 40 min/day). A standardized exercise test (SET) was performed 2 weeks before, and after L or UW training, and V180 bpm and La10.5 m/s calculated. Resting and post-SET gluteal and SDF muscle biopsies were obtained. Muscle [glycogen], [lactate] and [ATP], fiber type composition and min/max fiber diameters were analysed. Comparisons were made using GLM or ANOVA, mean ± SD and significance of $P<0.05$.

Results: Post-exercise SDF muscle lactate increased 3–4 fold to 30 ± 12 (UWT) and 41 ± 15 (LT) mmol/kg dw post-exercise. Post-exercise gluteal muscle lactate increased 2 fold to 55 ± 18 (UWT) and 62 ± 18 (LT) mmol/kg. No significant differences were found between UW and L post-exercise muscle [glycogen], [lactate] and [ATP], V180 or LA10.5 or fiber type composition. Gluteal fiber diameters decreased significantly with UW and L training. Fiber diameters in SDF muscle decreased with training to a significantly lesser extent after UW vs. L training.

Conclusions: At maximal speed, both SDF and gluteal muscle have a significant anaerobic response which was not impacted by UWT or LT training at a walk. UWT may be of benefit by attenuating the training induced decrease in fiber sizes in SDF muscle thereby enhancing SDF muscle strength.

Ethical Animal Research: All procedures were approved by the Institutional Animal Care and Use Committee of the University of Minnesota. **Sources of funding:** Supported by University of Minnesota Equine Center with funds provided by the Minnesota Racing Commission, University of Minnesota Agricultural Experiment Station, and private donors. **Competing interests:** none.

SUBCHONDRAL BONE REMODELLING IS MORE ACTIVE IN RESTING THAN TRAINING THOROUGHBRED RACEHORSES

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Introduction: Subchondral bone fatigue is an important cause of poor performance, morbidity and mortality in racehorses. Fatigued bone is repaired by remodelling, a process that is attenuated under high cyclical loads, yet the effects of training on remodelling activity in Thoroughbred racehorses is unknown. We hypothesised that remodelling rates are lower in training horses than those that are resting from training.

Methods: Sections of the palmar metacarpal condyles from Thoroughbred horses that were in race training (n = 24) or resting from

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training (n = 24) at the time of death were examined with light microscopy and back scattered electron microscopy (BSEM). Bone area fraction (B.Ar/T.Ar), and eroded bone surface (E.Pm/T.Ar) were measured within two regions of interest; (1) the lateral parasagittal groove (PS); and (2) the lateral condylar subchondral bone (LC). BSEM variables were analysed for the effect of group (independent samples t-test), region (paired t-test) and interaction with time since change in training status (general linear model). Mean ± SE are reported.

Results: For both regions of interest E.Pm/T.Ar was lower in the training horses (PS: 0.39 ± 0.06 mm^{-1}, LC: 0.24 ± 0.04 mm^{-1}) than in the resting group (PS: 0.65 ± 0.07 mm^{-1}, P = 0.010, LC: 0.85 ± 0.10 mm^{-1}, P<0.001). Lower subchondral bone porosity was observed, reflected by higher B.Ar/T.Ar in the LC of training horses (90.8 ± 0.6%) than resting horses (85.3 ± 1.4%, P = 0.0010). E.Pm/T.Ar was higher with increased duration of a training period in the lateral condyle (r^2 = 0.26, P = 0.011).

Conclusions: Race training inhibits remodelling in the subchondral bone of highly loaded areas of the distal metacarpus. Therefore periods of less intense loading are important for allowing repair of subchondral bone fatigue in Thoroughbred racehorses.

Ethical Animal Research: The study was performed on material collected during post-mortem examination. **Sources of funding:** Rural Industries Research and Development Corporation of the Australian Commonwealth Government and Racing Victoria Limited. **Competing interests:** none.

HIGH-RESOLUTION RESPIROMETRY MAY IDENTIFY EARLY INDICATORS FOR EXERTIONAL RHABDOMYOLYSIS IN STANDARDBRED RACEHORSES

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Introduction: During racing, mitochondrial oxidative phosphorylation (OXPHOS) is a major source of energy for muscle contraction. Assessment of muscle OXPHOS capacity by high-resolution respirometry (HRR) might identify animals with altered energy metabolism and consequently increased risk of exertional rhabdomyolysis (ER).

Methods: Prospective cohort study. Ten healthy French Standardbreds in active training, aged 4–7 years, recruited at the start of a competitive racing season, underwent a standardised field exercise test with determination of speed at onset of lactate accumulation (VLa4) and measurement of serum creatine kinase activity (CK) two hours post-exercise. *Triceps brachii* muscle microbiopsies were obtained for determination of OXPHOS and electron transfer system (ETS) capacities with HRR. Associations between HRR variables and occurrence of ER over the following four months of racing were analysed using univariate logistic regression.

Results: Two horses developed ER during the study period: one within one month of recruitment, another presented multiple minor episodes over the season. For these ER horses OXPHOS/ETS ratio was lower, and Complex I/Complex I + II ratio and Complex I /Complex II ratio were higher than non-affected horses in the study (perfect prediction of occurrence of ER with ratio of ≤0.74, ≥0.60 and ≥0.715 respectively). VLa4 for ER horses was within the range of non-affected horses. Post-exercise CK was within normal limits for all horses in the study.

Conclusions: OXPHOS and ETS capacities and their derived ratios indicate dysfunctional muscle energy metabolism whereas VLa4 and CK were unremarkable in horses subsequently affected by ER.

Ethical Animal Research: The study was approved by Liège University's Animal Ethics Committee (agreement N° 07-629); owner informed consent was obtained. **Sources of funding:** Part of the study protocol was funded by Les Haras Nationaux (France). **Competing interests:** The Oxygraph-2k HRR apparatus was provided on loan by OROBOROS Instruments (Innsbruck, Austria); there are no patents, products in development or marketed products to declare.

EXPRESSION AND REGULATION OF GLYCOGEN SYNTHASE IN EQUINE TYPE 1 POLYSACCHARIDE STORAGE MYOPATHY

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Introduction: Equine Type 1 polysaccharide storage myopathy (PSSM1) is associated with a missense, gain-of-function mutation in the equine skeletal muscle glycogen synthase gene (GYS1). Affected horses have excessive glycogen storage, amylopectate-like inclusions in their muscle and higher muscle glycogen synthase activities (GS) than controls. GS is normally allosterically-regulated by glucose-6-phosphate and enzyme activity is reduced by phosphorylation. We hypothesised that the higher GS activity in PSSM1-affected horses might be due either to 1) increased expression or reduced degradation of the mutant enzyme or 2) due to reduced phosphorylation.

Methods: Semimembranosus muscle samples were biopsied from 4 homozygotes (HH), 13 heterozygotes (RH) and 12 control (RR) Belgian Draught horses. Samples were analysed for glycogen content and GS activity. Western immunoblotting was used to examine expression of total and phosphorylated forms of GS, GSK3β and GLUT4. Data was analysed using non-parametric ANOVA statistical tests with post-hoc Dunn's multiple comparison tests.

Results: PSSM1 affected heterozygotes and homozygotes had significantly higher muscle glycogen contents than controls (RR vs. RH P = 0.005, RR vs. HH P = 0.041) and GS activity was significantly higher in PSSM1-affected homozygotes than in heterozygotes and control horses (P = 0.03). Although there was no difference in total GS expression between groups, there was significantly more expression of GS phosphorylated at site 2 + 2a in PSSM1-affected horses in comparison with controls (P = 0.009).

Conclusions: Our data suggest that increased GS activity in PSSM1-affected horses is not associated with increased expression or degradation of the mutant enzyme and occurs despite increased phosphorylation of GS at site 2 + 2a. Alternative mechanisms, including aberrant ligand binding might account for the increased GS activity in PSSM1-affected muscle.

Ethical Animal Research: Muscle biopsy samples were collected according to ethical approval from the local Institutional Animal Care and Use Committee. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Petplan Charitable Trust and The Royal Veterinary College. **Competing interests:** none.

IN SILICO THREE DIMENSIONAL MODELLING OF MUTANT GLYCOGEN SYNTHASE ASSOCIATED WITH EQUINE TYPE 1 POLYSACCHARIDE STORAGE MYOPATHY

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Introduction: Equine Type 1 polysaccharide storage myopathy (PSSM1) is associated with a dominant, missense mutation in the equine glycogen synthase gene (GYS1) that increases the enzyme's activity. The amino acid sequence surrounding the PSSM1 mutation is highly conserved amongst species from yeast to horses suggesting that this region plays an important role, but its functional significance has yet to be determined.

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Methods: Computer modelling can predict three dimensional (3D) protein structures using related proteins as templates. Yeast glycogen synthase (GS) has high sequence homology (73%) to the equine enzyme and since the yeast enzyme's structure has been solved, we hypothesised that in silico modelling based on the yeast protein using MODELLER and SwissPDBViewer software would predict the enzyme's structure and explain the mutant enzyme's increased activity.

Results: Homology-modelling revealed that, like the yeast enzyme, equine GS likely forms a tetramer surrounding a catalytic cleft and undergoes large structural rearrangements upon G6P binding to highly-conserved binding sites. The enzyme's glycogen binding sites are located on the extremities of the enzyme and are also conserved in the equine protein. The PSSM1 mutation lay close to the G6P binding site, but did not confer any clear structural rearrangement on the protein's 3D configuration. The mutation was distant from the known phosphorylation sites and the glycogen binding sites.

Conclusions: The close proximity of the mutation to the G6P binding site suggests that it might influence substrate interaction or allosteric regulation. Further studies evaluating G6P ligand binding and enzyme activation are warranted and might best be made following purification of the mutant and wild type proteins.

Ethical Animal Research: No animals were used in the study. **Sources of funding:** Petplan Charitable Trust and The Royal Veterinary College. **Competing interests:** none.

MITOCHONDRIAL RESPIROMETRY IN SKELETAL MUSCLE OF EXERCISING EVENTING HORSES RUN TO EXHAUSTION

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Introduction: Mitochondria play a central role in energy metabolism during muscle contraction and in the balance of anaerobic and aerobic energy production. This study evaluated mitochondrial respirometry in skeletal muscle of eventing horses before and after an intense effort.

Methods: Fourteen Brazilian Sport Horses 4 to 6 years old performed an incremental velocity test on a high speed treadmill inclined to 3% and galloped at speeds up to 8 m/s until exhaustion. Biopsies from the gluteus medius muscle were obtained before and immediately after the end of the test. Mitochondrial respiration was assessed by O2K oxygraph of high resolution (Oroboros). The results were standardized by minute and per gram of wet weight of muscle and expressed as mean ± SD. Plasma lactate was measured using a spectrophotometer. Results were compared by paired t–test ($P<0.05$).

Results: The maximal respiration value of mitochondria did not change in gluteus medius muscle of horses at exhaustion. However, there was an increase in stage 3 mitochondrial respiration with pyruvate/malate substrate, and also an increase of non-mitochondrial respiration after exercise. Lactate values before and after exercise were 0.4 ± 0.1 mmol/L and 12.1 ± 4.6 mmol/L, respectively.

Conclusions: There is little change in mitochondrial respirometry in the gluteus medius muscle after intense exercise indicating predominantly anaerobic metabolism in the muscle of the eventing horses, as supported by the high plasma lactate concentrations after the test. The increase of oxidase reactions after intense exercise is related to an increase in non-mitochondrial respiration.

Ethical Animal Research: All procedures were approved by the Board of Ethical Research of Universidade Federal Rural do Rio de Janeiro: number 264/2012. **Sources of funding:** Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq; PRONEX and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ. **Competing interests:** none.

EFFECT OF SEDATION AND EXERCISE ON PHENYLALANINE PHARMACOKINETICS IN HORSES

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Introduction: Measurement of incorporation of stable isotope-containing amino acids into muscle is the gold-standard technique for measuring muscle protein synthesis (MPS), but its use in assessing MPS in horses has not been evaluated. Determination of MPS requires steady-state tracer plasma enrichment and serial muscle biopsy. Given that MPS is often assessed in response to exercise and that muscle biopsy in the standing horse typically requires sedation, the influence of exercise and sedation on tracer kinetics was determined.

Methods: Three mature Thoroughbred geldings received 0.1 mg/kg D5-phenylalanine (D5-Phe) IV and plasma D5-Phe enrichment was measured over 3 hrs. Pharmacokinetic modeling determined the rate of appearance and phenylalanine pool size, from which a loading dose and infusion rate that would achieve a steady 5–10% enrichment were calculated. Subsequently, steady-state was confirmed by measuring plasma enrichment during 6 hours of infusion after loading. Horses then received the same dose and completed a 12-minute exercise test and were sedated with xylazine 0.4 mg/kg IV. Plasma D5-Phe enrichment was determined every 5–15 minutes for 2 hours after both exercise and sedation.

Results: The mean rate of phenylalanine appearance in the horse was 56 umol/kg/hr (SD 24) and mean phenylalanine pool size 41 umol/kg (SD 8.8). A loading dose of 3.3 umol/kg and an infusion rate of 4.5 umol/kg/hr D5-phenylalanine was calculated and subsequently, confirmed to achieve steady state tracer concentration at 5.6–9.1% APE in sedentary horses. Exercise caused a transitory, and sedation a more prolonged reduction in plasma enrichment (up to 20%).

Conclusions: D5-phenylalanine is a suitable metabolic tracer in the horse as a steady-state can be achieved, however interventions should be taken into account.

Ethical Animal Research: The study was performed in in accordance with the local Ethics Committee approval and Home office project license (PPL 70/7523) under the UK A(SP)A Act 1986. **Sources of funding:** Boehringer Ingelheim. **Competing interests:** R Naylor is completing a PhD studentship sponsored by Boehringer Ingelheim.

CAN STALLED HORSES PROVIDED LIGHT EXERCISE AND "WEEKEND" TURNOUT MAINTAIN BONE MINERAL CONTENT?

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Introduction: Previous research suggests stalling may have a negative influence on bone mineral content (BMC). Yet slow, long-distance exercise has not been successful in improving BMC compared to free-choice exercise. Still, it is a common practice to stall horses with light exercise and minimal turnout. We hypothesized stalled horses, despite "weekend" turnout and light exercise, would have lower estimated BMC compared to pasture turnout.

Methods: Thirteen mature horses were split into two groups, Control (C, n = 6) and Stalled (S, n = 7). Digital radiographs were taken of the third metacarpal for estimation of BMC by radiographic bone aluminum equivalence (RBAE). After a 42-d period (P1) additional radiographs were taken and S-horses stalled for 4-d (receiving light under-saddle exercise), followed by 72-h pasture turnout. This pattern repeated for an additional 42-d (P2) followed by radiographs. Radiographs were analyzed for optical

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density of bone cortices and total BMC compared to an aluminum step-wedge on each cassette. Changes in BMC were analyzed using PROC mixed in SAS with repeated measures.

Results: No treatment differences were observed in the medial (P = 0.72) or lateral (P = 0.46) cortices. Dorsal and palmar cortices exhibited a treatment by period interaction (P<0.01, P<0.02), remaining unchanged in C but S decreased over P1 and returned to baseline in P2. Total RBAE was not different by treatment or period.

Conclusions: Contrary to our hypothesis, horses stalled with light exercise and "weekend" turnout maintained BMC compared to themselves and controls at pasture. Such management may be considered for maintaining BMC when horses must be stalled for weekday riding or training. Further research is necessary to determine minimum turnout and exercise needs to maintain BMC when stalling.

Ethical Animal Research: The study was approved by the Middle Tennessee State University Institutional Animal Care and Use Committee **Sources of funding:** The Horse Science Program and Office of Research at Middle Tennessee State University. **Competing interests:** none.

FATIGUE IN THE SUPERFICIAL AND DEEP DIGITAL FLEXOR MUSCLES DURING EXERCISE IN THOROUGHBRED HORSES

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Introduction: One of the causes of injury to the superficial digital flexor tendon (SDFT) is assumed to be repeated high intensity loading of the tendon. Earlier fatigue of the deep digital flexor muscle (DDFM) in comparison to the time of fatigue of the superficial digital flexor muscle (SDFM) is also assumed to increase load on the SDFT during long distance running. This study evaluated the fatigue of SDFM and DDFM with maximal exercise.

Methods: Two wire electrodes were inserted into the SDFM and DDFM of 6 Thoroughbred horses (461–557 kg). Electromyogram (EMG) and hoof strain gauge signals were recorded at 2,500 Hz with horses on an inclined treadmill (6%), and filter processing was applied (high-pass 10 Hz for EMG and low-pass 200 Hz for strain gauge). Integrated EMG (iEMG), the median frequency (MF) of EMG, and the stride frequency (SF) were compared using paired t-test during 100–105% heart rate max exercise and trotting before and after maximal exercise.

Results: The SF significantly decreased and iEMG of SDFM tended to decrease during maximal exercise (P = 0.055), while MF of EMG in SDFM and DDFM did not change. The iEMG of SDFM and DDFM significantly decreased and MF of EMG in DDFM tended to decrease at a trot after maximal exercise (P = 0.063).

Conclusions: The DDFM may fatigue earlier than SDFM during long distance running. Further study is needed to evaluate the relationship between the decrease in iEMG of SDFM and DDFM during maximal exercise and the injury to SDFT.

Ethical Animal Research: The study was approved by the Animal Welfare and Ethics Committee of the Japan Racing Association Equine Research Institute. **Sources of funding:** Japan Racing Association. **Competing interests:** none.

INFLUENCE OF WEIGHT LOSS ON SKELETAL MUSCLE MITOCHONDRIAL FUNCTION IN THE MATURE HORSE

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Introduction: Obesity creates many metabolic issues in horses, yet stepwise alterations in mitochondrial capacity and oxidant status during weight loss have not been assessed. Skeletal muscle contains subsarcolemmal (SSM) and interfibrillar (IFM) mitochondria, which respond differently to physiological stimuli, impacting tissue and systemic oxidant status. It was hypothesised that horses would display improved muscle mitochondrial subpopulation function in middle gluteal muscle, and decreases in circulating oxidant status markers during weight loss from an obese (7 to 8) to moderate (5) body condition score (BCS).

Methods: 8 light-type horses were studied. Mitochondrial subpopulations were isolated through differential centrifugation and all enzymatic activity through colorimetric kinematic assays. Effects of BCS reduction or percent weight loss were evaluated using SAS (mixed procedure with repeated measures).

Results: Plasma nitrate decreased in response to BCS reduction (P = 0.06) and percent weight loss (P = 0.06), whereas erythrocyte total glutathione (P = 0.06) concentration increased with decreasing BCS. Mitochondrial electron transport chain complexes I and IV displayed greater activity in SSM than IFM (P≤0.03), while all complexes in IFM had decreased activity due to both weight parameters (P≤0.01). Interactions between subpopulation complex IV activity and weight loss markers (P<0.05) were displayed. Citrate synthase activity, indicating mitochondrial number, was greater in SSM than IFM (P<0.0001) but was unaffected with weight loss. Lipid peroxidation was decreased with BCS change (P = 0.01) and weight loss (P = 0.02), displaying greater amounts in SSM than IFM (P≤0.02).

Conclusions: Alterations in complex activities and lipid peroxidation suggest IFM are more affected by weight loss, with large contributions from complex IV by-products. Mitochondrial component flexibility may contribute individually to disease development and performance of equine athletes.

Ethical Animal Research: The study was approved by the Middle Tennessee State University Institutional Animal Care and Use Committee. **Sources of funding:** West Virginia University Summer Undergraduate Research Experience Program and Middle Tennessee State University. **Competing interests:** none.

NUTRITION

THE USE OF NUTRITIONAL SUPPLEMENTS IN DRESSAGE AND EVENTING HORSES

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Introduction: Selecting the appropriate nutritional supplements for performance horses is difficult, due to significant gaps in the current scientific evidence. Our hypothesis was that nutritional supplements are chosen based on owner's perception of discipline specific health and performance problems.

Methods: A mixed methods (open and closed questions) online questionnaire was distributed through British Eventing and Dressage

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websites, to collect data on demographics of owners and their horses, supplements used, and their opinion on health and performance problems in their discipline, and in their own horses. Data was evaluated using descriptive analysis and Sign and Fisher's exact tests for quantitative data, and thematic analysis for qualitative data.

Results: 599 responses met the inclusion criteria, of which 441 were or had competed in dressage, and 158 in eventing (unaffiliated novice to affiliated advanced levels). Participants had been riding for 26.4 (3–60) (mean (range)) years, currently competed 1.2 (0–10) horses, and used 2 (0–12) supplements in their top horse. Participants ranked the most important health and performance issues as (1) Energy and behaviour, (2) Lameness, (3) Back and muscle problems for dressage, and (1) Lameness, (2) Energy and behaviour, (3) Stamina and fitness for eventing horses. The most common reason supplements were used in individual horses were (1) Joints and mobility, (2) Behavioural problems for dressage, and (1) Electrolytes, (2) Joints and mobility for eventing.

Conclusions: There was discrepancy between participants' opinions of the main health and performance problems within their discipline, and their reasons for using supplements.

Ethical Animal Research: The study was approved by the School of Veterinary Medicine and Science Ethics Committee at University of Nottingham. **Sources of funding:** School of Veterinary Medicine and Science, University of Nottingham. **Competing interests:** Teresa Hollands is employed by Dodson and Horrell Ltd.

EFFECT OF CHELATED CHROMIUM AND L-CARNITINE ON SUBCUTANEOUS FAT DEPOSITION IN THE MANGALARGA MARCHADOR BREED AFTER TRAINING

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Introduction: The Brazilian Mangalarga Marchador breed is valued for its gait. However few studies have assessed the use of nutritional additives on this breed's performance. This study was designed to test the hypothesis that chelated trivalent chromium and L-carnitine feed supplementation may reduce subcutaneous fat deposition after training.

Methods: 13 mares (2.5 to 3 years old) were assigned to one of three treatments (T1, 10 mg Cr/day; T2, 10 g LCN/day; T3, control) according to a completely randomized design, with split-plot arrangement. The diet was Panicum maximum cv. Massai and concentrate (50:50 ratio and 2.5% BWT). During 42 days the mares received supplementation and exercised for 50 min on a treadmill and 60 min on an automatic walker every other day, resting on Sundays. Subcutaneous fat was measured with ultrasound before and after training in the back-loin(GDL), croup(GG), and base of the tail(GBC). Results were compared by Tukey test ($P<0.05$).

Results: Before training, the variables were similar across the groups ($P>0.05$). After training, in GDL, T2 (1.76 mm) was 37.37% lower ($P<0.05$) than control (2.81 mm), while T1 (2.22 mm) was similar to T2 and control. There was no difference between treatments ($P>0.05$) in GG. In GBC, there was a 24.45% increase from pre (8.59 mm) to post-treatment (10.69 mm) evaluations, regardless of the treatment.

Conclusions: Supplementation with L-carnitine reduces subcutaneous fat deposition in the lumbar region of Mangalarga Marchador mares after training, which may be indicative of increased use of subcutaneous fat in this region as a substrate for exercise.

Ethical Animal Research: Procedures were approved research by the University (no. 237 / 2012 protocol). **Sources of funding:** CNPq, CAPES, FAPEMIG, ABCCMM, Lab TORTUGA, Haras Esperança e Grande Sertão. **Competing interests:** none.

EFFECT OF DIET AND ROAD TRANSPORT ON POST-EXERCISE PLASMA LACTATE CONCENTRATION

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Introduction: Horses are usually transported before competitions. The aim of this study was to compare the lactate response in horses on two diets that were transported or not transported before exercise.

Methods: Six trained and raced Standardbred trotter mares, all accustomed to transportation, were used. Two diets were fed in two periods of 29 days in a crossover design; one forage-only diet (F) and one diet with 50% of DM intake from forage and 50% from oats (FO). The horses had slow exercise 1–3 times/week (approximately 1 h walk and slow trot [6–7 m/s]) and intensive exercise1–2 times/week (4000 m slow trot warm-up, 2000 m at 10–11 m/s on a race track or five 500 m intervals at 9 m/s on a slope, slow trot downhill). At day 21, horses were subjected to transport for 100 kilometres before they performed an exercise test (TT) (4000 meter warm-up [6.3–10 m/s] and 2140 m "race" [11.6–12.7 m/s]). At day 26, the horses performed a control test (CT) where they were kept in their stall before the exercise test was performed. Blood samples were collected after the finish line and 10 and 30 min post-exercise. Plasma lactate (Lac) was analysed using a spectrophometric method. ANOVA was performed using PROC MIXED (significant difference $P<0.05$).

Results: There was no difference in Lac (after finish and 10 and 30 min post-exercise) between diets (F: 14.9 vs. FO: 17.1 mmol/l SEM: 2.1) or exercise tests (TT: 15.9 vs. CT: 16.1 mmol/l SEM 2.0).

Conclusions: Diet and transport seem not to affect post-exercise plasma lactate in experienced competition horses transported 100 km.

Ethical Animal Research: The Umeå local ethics committee approved this study. **Sources of funding:** KSLA (The Royal Swedish Academy of Agriculture and Forestry). **Competing interests:** none.

FACTORS AFFECTING OWNERS' CHOICE OF NUTRITIONAL SUPPLEMENTS IN DRESSAGE AND EVENTING HORSES

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Introduction: Horse owners are faced with a vast range of supplements and different sources of information. The aim of this study was to evaluate sources of information used by owners/riders when choosing supplements for competition horses, their opinions on different sources of information and evaluation of factors that affect their decisions.

Methods: A mixed methods (open and closed questions) online questionnaire was distributed through British Eventing and Dressage websites to owners/riders of dressage and/or event horses, to collect data on the demographics of the owner/rider and their horses, sources of information used when choosing nutritional supplements, and their opinion on these different sources. Data was evaluated using descriptive analysis and Chi-squared tests for quantitative data, and thematic analysis for qualitative data.

Results: 757 responses met the inclusion criteria, and owners/riders competed at dressage and eventing from unaffiliated novice to affiliated advanced level. Veterinary surgeons were considered the most reliable source of information, followed by nutritionists, research studies,

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coach/trainer and other horse owners. 'Other horse owners' were the top source (18% of participants) for the participants' most recent choice of supplement. Respondents with a higher education qualification were significantly more likely to use research papers as a source of information (P<0.005). Owners/riders under 34 years of age were significantly more likely to use a coach / trainer as a source of information (P<0.005).

Conclusions: Horse owners identified veterinary surgeons, nutritionists and research papers as reliable sources of information, but ultimately most chose their supplement based on recommendations of other owners or coaches/trainers.

Ethical Animal Research: The study was reviewed and approved by the School of Veterinary Medicine and Science Ethics Committee at the University of Nottingham. **Sources of funding:** School of Veterinary Medicine and Science at the University of Nottingham. **Competing interests:** Teresa Hollands is employed by Dodson and Horrell Ltd.

ENERGY EXPENDITURE DURING A 160 KM ENDURANCE RACE

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Introduction: In endurance racing, horses are required to complete distances of up to 160 km in a single day. To date, the energy expenditure (EE) of such effort has not been quantified. This study aimed to evaluate EE during a 160 km endurance race from heart rate (HR) monitoring.

Methods: Three weeks before competing, two endurance horses (H1 and H2) were submitted to a field exercise test to determine their individual VO2-HR relationship. VO2 and HR were recorded simultaneously at trot (13–14 km/h for 10 min), and at canter (18–19 km/h for 10 min, and 22–23 km/h for 5 min), using the equine K4b2, a portable metabolic measurement system. H1 was then enrolled in Monpazier CEI*** and H2 in Florac CEI***. HR was monitored from 2 minutes before the start to the finishing line of each loop. EE of exercise was calculated from individual VO2-HR relationship and caloric equivalent of oxygen.

Results: H1 and H2 completed the race at an average speed of 17.02 and 14.46 km/h, respectively. Mean HR was 108 ± 8 and 124 ± 24 and peak HR was 169 and 204 for H1 and H2, respectively. The proportion of time spent at HR≥180 was 0 and 2.7% for H1 and H2 respectively. EE of exercise was evaluated at 177 and 240 MJ for H1 and H2 respectively.

Conclusions: A 160-km endurance race might present a considerable energy cost, at least 5 times the EE of non-working state.

Ethical Animal Research: The project was conducted under license from the Department of Health and Animal Care of the French Veterinary Authority (no. A21002) and ethics committee approval n° B0710. **Sources of funding:** Burgundy Region and AgroSup Dijon. **Competing interests:** none.

A COMPARISON OF RACING RESULTS BETWEEN STANDARDBRED HORSES FED A FORAGE-ONLY DIET AND THEIR SIBLINGS

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Introduction: High concentrate diets increase the risk of several health problems and high energy forage diets could therefore be an alternative. The aim of this study was to compare the number of horses and the number of races performed at the age of three by a group of 16 Standardbred horses fed a forage only-diet from the age of 1.5 years (F) with the results of their siblings (S).

Methods: To F-horses, a forage diet was offered ad libitum (10.2–11.7 MJ metabolisable energy, 10–16% crude protein/kg dry matter) and supplemented daily with minerals and vitamins to meet requirements. F-horses were trained by pupils at the National Trotting School during supervision of professional trainers. For each individual in the F-group, the mean racing results from a maximum of five older siblings (same mother) were used for the S-group (5 siblings, n = 9; 4-3 siblings, n = 4 and 1–2 siblings, n = 3). The siblings selected were born closest in time in relation to the F individual. S-horses were trained by different trainers and it was assumed that they had been fed a conventional high concentrate diet. Racing results were obtained from the Swedish Trotting Association. ANOVA and a Tukey test were used to analyse differences between groups.

Results: There was no difference in the number of horses that competed (F: 56% vs S: 54%, SE 10) and in the number of races performed (F: 1.6 vs S: 2.9, SE 0.5).

Conclusions: The study indicates that a forage-only diet may not be a limitation to achieve conventional training results and could therefore be an alternative to conventional diets.

Ethical Animal Research: The study was approved by the local ethics committee in Umeå, Sweden. **Sources of funding:** Swedish University of Agricultural Sciences, the Swedish National Trotting School at Wången, the Swedish Trotting Association, The Swedish Horse Council Foundation, the Swedish Horse Racing Totalisator Board (ATG), Trioplast AB and Dow Chemicals. **Competing interests:** none.

OIL SUPPLEMENTATION PRODUCES AN INCREASE IN ANTIOXIDANT BIOMARKERS IN FOUR-BEAT GAITED HORSES

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Introduction: Marcha is typically a low intensity/medium duration exercise that has the potential to induce oxidative stress. The objective of the present study was to test the hypothesis that supplementation with oil would alter biomarkers of oxidative stress in four-beat gaited horses during marcha training.

Methods: Eleven gaited horses underwent training for 60 min (~60% consisting of marcha at ~3.5 m/s) 3 d/wk for eight weeks. Subjects were fed elephant grass (~15 kg/day) and concentrate (~5 kg/day). They were also supplemented with 300 mL of oil per os (vitamin E: 300 IU/kg, omega-3: 300 g/kg; omega-6: 80 g/kg) and had free access to water and salt. Blood samples were collected before and after four and eight weeks of supplementation for the measurement of GPx, SOD, RBC, hemoglobin, hematocrit, WBC, TPP, urea, creatinine and uric acid. ANOVA and Tukey tests were used for statistical analysis (P<0.05).

Results: Increases in GPx (~12%), SOD (~51%) and uric acid (~19%) were observed after 8 week of supplementation (P<0.05). There were changes (P<0.05) in [urea] and [creatinine]; however, there were no changes (P>0.05) in RBC, hemoglobin, hematocrit or WBC. Published research has demonstrated that administration of supplements with antioxidants enhances the antioxidant system and protects against oxidative stress during exercise. Supplementation with oil, rich in vitamin E, omega-3 and -6, produced a beneficial increase in antioxidant capacity in gaited horses during training.

Conclusions: The elevation in [GPx], [SOD] and [uric acid] in the present study offers a strategy to counterbalance the physiologic processes that overwhelm endogenous antioxidants during training.

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Ethical Animal Research: Methods were approved by UFRPE Animal Care Unit (#026/2013). **Sources of funding:** INTEGRALMIX Animal Nutrition (Fortaleza-CE), Haras Cascatinha (Camaragibe-PE) and CAPES. **Competing interests:** none.

EFFECT OF NON-STRUCTURAL CARBOHYDRATE, FAT AND FIBER INTAKE ON GLYCOGEN REPLETION FOLLOWING INTENSE EXERCISE

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Introduction: Muscle glycogen is a potentially limiting substrate for horses during intense exercise. Low-starch, high-fat concentrates have become popular for performance horses, but their effect on muscle glycogen usage and repletion is unclear.

Methods: Six Thoroughbred horses were studied in a 3 x 3 Latin Square design to measure the effect of non-structural carbohydrate (NSC), fat and fiber intake on glycogen repletion following intense exercise. Horses were fed isocaloric, isonitrogenous rations that supplied high (HS), medium (MS) or low (LS) NSC intake. The horses were fed 1.25% BW/d grass-hay and 1.0% BW/d concentrate. These rations provided 45%, 36% and 18% of total digestible energy (DE) from NSC, 11%, 15% and 23% of total DE from fat, and 30%, 33% and 45% of total DE from fiber. During each 1-month period horses were trained for three weeks on a high-speed treadmill followed by a three-day glycogen-depletion period comprising multiple bouts of intense exercise. Muscle biopsies were taken before and 0, 24, 48 and 72 hours post-depletion. A standardized exercise test (SET) was performed on day 1 of the depletion period.

Results: During the SET, lactate was lower in LS compared to MS or HS ($P<0.05$). Heart rate, plasma glucose, VO2, VCO2 were unaffected by treatment. Muscle glycogen depletion averaged 33%, 30%, and 36% in the HS, MS and LS treatments. Muscle glycogen repletion was significantly lower in the LS group 72 hours post exercise ($P<0.05$).

Conclusions: Low NSC concentrates may not provide enough substrate for glycogen repletion following multiple bouts of intense exercise.

Ethical Animal Research: Approved by Kentucky Equine Research animal use committee. **Sources of funding:** Kentucky Equine Research and Cargill. **Competing interests:** Some of the concentrates tested are owned by the funding sources. Dr Pagan is president of Kentucky Equine Research.

EFFECTS OF DIETARY SELENIUM AND TRAINING ON OXIDATIVE STRESS IN YOUNG HORSES

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Introduction: Exercise training can enhance antioxidant status, but it is unknown whether selenium supplementation can further improve antioxidant defenses.

Methods: American Quarter Horse yearlings (n = 20; mean ± SE 18 ± 0.2 months) balanced with regard to age and sex were randomly assigned to four dietary Se and training treatments for 14 weeks: 0.1 mg Se/kg DM with (n = 5) or without (n = 5) training or 0.3 mg Se/kg DM with (n = 5) or without (n = 5) training. Trained horses were exercised 4 d/wk for 30 min/d. At wk 14, all horses underwent a 2-hr, 27.5 km submaximal exercise test (SET). Blood samples and biopsies of the gluteus medius were obtained before and after training and in response to the SET. Serum Se, glutathione peroxidase (GPx) activity in whole blood and muscle, and plasma lipid hydroperoxides (LPO) were evaluated. Data were analysed using a mixed model ANOVA with repeated measures.

Results: Serum Se was higher ($P = 0.02$) at wk 14 in trained horses receiving 0.3 than 0.1 mg Se/kg, but was unaffected by diet in untrained horses. Muscle GPx activity was unchanged after 14-weeks of Se and training, but in response to the SET, muscle GPx was higher ($P = 0.05$) in trained than untrained horses. An effect of training*time ($P = 0.008$) was noted for whole blood GPx, reflecting greater ($P = 0.01$) activity in trained than untrained horses immediately after the SET. Plasma LPO was not influenced by dietary Se, but increased ($P<0.0001$) in untrained horses immediately after the SET while remaining unchanged in trained horses.

Conclusions: Training appears to mitigate oxidative damage in response to prolonged exercise in young horses. Although trained horses responded to a higher level of dietary Se, it showed no clear advantage for reducing oxidative stress.

Ethical Animal Research: This project was approved by the Institute of Food and Agricultural Sciences Animal Care Committee at the University of Florida (approval number 016-12ANS). **Sources of funding:** Partial financial support of this project was provided by Alltech, Nicholasville, KY, USA. **Competing interests:** none.

EFFECTS OF DIETARY SELENIUM AND TRAINING ON MITOCHONDRIAL FUNCTION IN YOUNG HORSES

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Introduction: Exercise training results in improved mitochondrial efficiency but it is unclear if dietary selenium level can further modify mitochondrial function.

Methods: American Quarter Horse yearlings (n = 20; mean ± SE 18 ± 0.2 months) balanced with regard to age and sex were randomly assigned to four dietary Se and exercise training treatments for 14 weeks: 0.1 mg Se/kg DM with (n = 5) or without (n = 5) training or 0.3 mg Se/kg DM with (n = 5) or without (n = 5) training. Trained horses were exercised 4 d/wk for 30 min/d. At week 14, all horses underwent a 2 hr, 27.5 km submaximal exercise test (SET). Permeabilized muscle fibers obtained from the gluteus medius of horses at rest were analysed for mitochondrial function via high resolution respirometry. Mitochondrial oxidative phosphorylation capacity with Complex I substrates was determined using the respiratory control ratio (RCR) of state 3 (with ADP) and state 2 (without ADP; leak respiration). Electron transport system capacity (ETSC) was determined by uncoupling phosphorylation and respiration using carbonyl cyanide p-trifluoromethoxyphenylhydrazone. Cytochrome c oxidase (CCO) and citrate synthase (CS) activities were assessed in resting muscle samples obtained at week 0 and 14, and in response to the SET. Data were analysed using a mixed model ANOVA.

Results: Leak, state 3, ETSC, CS and CCO were higher ($P<0.05$) at wk 14 compared to wk 0 in all horses, but were not affected by training or Se level. Additionally, CS and CCO were unaffected by the SET.

Conclusions: Mitochondrial number (CS activity) and efficiency were unaffected by training and dietary Se level, but did increase over time, perhaps due to growth of horses. A more strenuous training protocol may be needed to induce mitochondrial adaptations.

Ethical Animal Research: This project was approved by the Institute of Food and Agricultural Sciences Animal Care Committee at the University of Florida (approval number 016-12ANS). **Sources of funding:** Partial financial support of this project was provided by Alltech, Nicholasville, KY, USA. **Competing interests:** none.

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GENOMICS, PROTEOMICS, AND METABOLOMICS

A PILOT STUDY ON GENETIC SPEED MARKERS IN TURKISH THOROUGHBREDS

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Introduction: The 227-bp SINE insertion in the promoter region of the MSTN gene, the intronic single nucleotide polymorphism (SNP) of MSTN, g.66493737C>T, and the SNPs BIEC2-417274 and BIEC2-417495 located upstream and downstream of MSTN seem appropriate QTL markers for racing performance of elite Thoroughbreds. This pilot study aimed to find if these markers of racing ability can be used to optimize the management of the racing careers of Turkish Thoroughbreds.

Methods: Hair roots were used for DNA extraction. The promoter insertion was analysed by agarose gel electrophoresis of the PCR product. The SNPs were typed by the amplification refractory mutation system-real-time PCR. Best racing distance was defined as the distance of the races in which the horse obtained the best results. Results were semi quantified by a point score based on wining and places.

Results: BIEC2-417274, BIEC2-417495 SNP variants and the 227-bp SINE were strongly associated with g.66493737C>T. For the latter, the genotype frequencies were: C/C 0.23, C/T 0.52 and T/T 0.25. In total 39% of the Turkish races are distances >1600 m. Analysis of the racing career showed that both the sprinters (C/C) and the medium distance horse (C/T) were not entered optimally in races that fit with their genetic ability.

Conclusions: Turkish race programing should create more opportunities for the g.66493737 C/T genotypes to show their ability. This implies that more 1700 to 2800 m races should be scheduled in the racing calendar.

Ethical Animal Research: Owner informed consent was obtained. **Sources of funding:** Jockey Club of Turkey. **Competing interests:** none.

A SINGLE NUCLEOTIDE POLYMORPHISM (BIEC2-808543) ON ECA3 IS ASSOCIATED WITH RECURRENT LARYNGEAL NEUROPATHY INDEPENDENT OF HEIGHT IN THOROUGHBRED HORSES

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Introduction: Recurrent laryngeal neuropathy (RLN) is considered height-linked in Thoroughbreds. A SNP (BIEC2-808543) proximal to the *LCORL/NCAPG* gene significantly influences height variation in horses. It has been suggested that the C-allele of this SNP is associated with RLN risk. Our objective was to investigate the association between the BIEC2-808543 C-allele, RLN and height in a cohort of Thoroughbreds.

Methods: Resting and exercising upper respiratory tract endoscopic videos, laryngeal ultrasonograms, wither height, age and sex were recorded in 79 Thoroughbreds (age 34 ± 11 months). The Havemeyer grading scale was used to characterise RLN cases (Grade B or C at exercise) and controls (Grade 1.1 or 2.1 at rest, Grade A at exercise, normal laryngeal ultrasound). All horses were genotyped for the BIEC2-808543 SNP using a custom TaqMan SNP Genotyping assay. Logistic regression was performed measuring additive effects of allele dosage with sex, age and height as covariates ($P \leq 0.05$). Horses were subcategorised into tall (T,≥162 cm) and short (S,<162 cm) groups for C-allele frequency determination, with data presented as mean ± SD.

Results: There were 16 RLN cases (height 164.5 ± 4 cm) and 32 controls (height 156.2 ± 4 cm). With sex and age as covariates, a significant association between the C-allele and RLN cases was observed ($P = 0.01$). With height included as a covariate, a strong trend of association between the C-allele and RLN remained ($P = 0.06$). C-allele frequency was 41.7% for the T-RLN group (n = 12, height 166.2 ± 3 cm), 12.5% for the S-RLN group (n = 4, height 159.5 ± 2 cm), 13% for the T-Control group (n = 16, height 165.1 ± 2 cm) and 14% for the S-Control group (n = 16, height 156.2 ± 4 cm).

Conclusions: The association between the BIEC-808543 C-allele and RLN appears to be independent of height.

Ethical Animal Research: Institutional Animal Research Ethics Committee approval was obtained. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Science Foundation Ireland under Grant Number 11/PI/1166. **Competing interests:** Dr Hill is a co-founder and Chairman of Equinome Ltd.

INVESTIGATION OF *MSTN* GENOTYPE ASSOCIATION WITH VO_{2PEAK} IN UNTRAINED YEARLINGS

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Introduction: In Thoroughbreds, genetic variation in myostatin (*MSTN* g.66493737C/T) is highly predictive of optimum race distance and influences mRNA expression, precocity, skeletal muscle development, two-year-old earnings, speed variables and skeletal muscle fibre type. The aim of this study was to investigate *MSTN* variation associations with VO_{2peak} in untrained yearling Thoroughbreds.

Methods: Peak oxygen consumption (VO_{2peak}) was measured in 41 untrained Thoroughbred yearlings (16 colts, 25 fillies, 19.8 ± 1.4 months) from one flat racing yard (November-December). Data was collected as horses freely travelled up an all-weather gallop (2% incline) with no rider. VO_2 was measured using an ergospirometry and oximetry mask and associated customized software validated for field testing. Horses were instrumented with a GPS-HR monitor, light-weight mask and data-capture equipment. HRpeak, maximal velocity (Vmax) and exercise distance were recorded. *MSTN* genotypes were determined using a customised Taqman SNP genotyping assay. Tests of association between genotype and phenotype were performed using PLINK software.

Results: Average exercise distance was 1700.5 ± 33.4 m, Vmax 15.0 ± 1.4 m/s, and HRpeak 221.2 ± 9.6 bpm. *MSTN* genotypes and VO_{2peak} based on 6 breaths were n = 16 CC, VO_{2peak} = 119.49 ± 21.8; n = 22 CT, VO_{2peak} = 116.40 ± -27.2; n = 3 TT, 115.08 ± 20.2.There was no association ($P>0.05$) between genotype and VO_{2peak}.

Conclusions: In yearling Thoroughbreds no association between *MSTN* genotype and untrained peak oxygen consumption was observed. This may be a function of the small number of TT genotype individuals evaluated or a reflection of a requirement for *MSTN*-associated skeletal muscle entrainment in VO_{2peak} changes resulting from a period of exercise training. Such changes may be associated with *MSTN* genotype and this requires further investigation.

Ethical Animal Research: Institutional Animal Research Ethics Committee approval was obtained. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Science Foundation Ireland under Grant Number 11/PI/1166. **Competing interests:** Dr Hill is a co-founder and Chairman of Equinome Ltd.

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CANDIDATE GENE SNP ASSOCIATIONS WITH FAT-FREE MASS (FFM) MEASUREMENTS IN HORSES-IN-TRAINING

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Introduction: Body composition including percentage of fat, fat mass and fat-free mass (FFM) is an important consideration for athletes. An evaluation of FFM in racehorses concluded that racing performance is positively correlated with FFM both pre- and post-training. Athletic performance is influenced by interactions among the environment and genes that influence anatomical, metabolic and physiological traits relevant to exercise. The aim of this study was to determine the effects of genetic variation in six candidate genes with functions in growth and metabolism (*IL1RA, PTPN1, MC3R, IGF1, LEPR* and *MSTN*) on FFM in Thoroughbreds.

Methods: FFM was calculated from rump fat thickness measured ultrasonographically in 110 two-year-old Thoroughbred horses-in-training in the same yard after two (T2) and eight (T8) months of training. Twenty-one SNPs in six genes were selected from the EquCab2.0 SNP database and were genotyped for all horses using custom Sequenom SNP genotyping assays. Quantitative tests of association were performed between SNPs and FFM at T2 and T8, and the FFM ratio between the two time points (T2 : T8) using PLINK software.

Results: Two SNPs in both the *IGF1* (Insulin-Like Growth Factor 1) and *PTPN1* (Protein Tyrosine Phosphatase, Non-Receptor Type 1) genes were significantly associated with FFM (T2) ($P<0.05$). The two *IGF1* SNPs and two *LEPR* (Leptin Receptor) gene SNPs were significantly associated with FFM (T8) ($P<0.05$). One *LEPR* SNP was significantly ($P<0.005$) associated with changes in FFM (T2 : T8).

Conclusions: These results suggest a genetic contribution to FFM variation in Thoroughbreds. Further validation will determine whether these SNPs may be used to predict the effects of training on FFM.

Ethical Animal Research: Institutional Animal Research Ethics Committee approval was obtained. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** The study was funded by a Science Foundation Ireland, President of Ireland Young Researcher Award (04-YI1- B539). **Competing interests:** Dr Hill is a co-founder and Chairman of Equinome Ltd.

EFFECT OF TRAINING ON *PPARGC1A* AND *FNDC5* GENE EXPRESSION IN THOROUGHBRED HORSES

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Introduction: PGC-1α and irisin, encoded by the *PPARGC1A* and *FNDC5* genes, play key roles in mediating the beneficial effects of exercise. *PPARGC1A* gene expression is induced by exercise and has been shown to upregulate *FNDC5* expression. Higher levels of *FNDC5* have been observed in young male human athletes compared to sedentary controls. *FNDC5* gene expression has also been shown in some species to be modulated by myostatin (*MSTN*), a growth factor that limits muscle hypertrophy. In horses *MSTN* variation (g.66493737C>T) affects gene expression and racing performance. Thoroughbreds homozygous for the C-allele have significantly higher levels of *MSTN* mRNA than homozygous T : T horses. The aim of this study was to investigate the responses of *PPARGC1A* and *FNDC5* in skeletal muscle to exercise and investigate the relationship with *MSTN* genotype.

Methods: Skeletal muscle (gluteus medius) biopsies were taken from untrained yearling Thoroughbreds ($n = 27$) at rest and after a ten month period of training at two-years-old. Skeletal muscle gene expression was measured using real-time qRT-PCR assays and data was analysed using qBase software.

Results: A significant increase in both *PPARGC1A* ($P = 0.0001$, $FC = 1.82$) and *FNDC5* ($P = 0.039$, $FC = 1.72$) was observed post-training. A significant positive correlation was found between *FNDC5* and *PPARGC1A* expression levels post training ($P = 0.01$, $R2 = 0.22$). There was no relationship between *PPARGC1A* or *FNDC5* gene expression and *MSTN* genotype.

Conclusions: These data indicate a coordinated role for PGC-1α and irisin in the skeletal muscle response to exercise in Thoroughbred horses. Their known roles in mitochondrial biogenesis and brown fat metabolism suggest important roles in the adaptive response to exercise, which is independent of *MSTN*-determined muscle type.

Ethical Animal Research: Institutional Animal Research Ethics Committee approval was obtained. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Science Foundation Ireland under Grant Number 11/PI/1166. **Competing interests:** Dr Hill is a co-founder and Chairman of Equinome Ltd.

FAECAL MICROBIOME OF THE THOROUGHBRED RACEHORSE AND ITS RESPONSE TO DIETARY AMYLASE SUPPLEMENTATION

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Introduction: The horse has evolved to survive on a low-energy, high-fibre diet. The addition of starch, in the form of cereals, to the diet of racehorses to satisfy the high energy demands of extreme exercise has been identified as a risk factor for intestinal dysfunction. In this study we use 454 pyrosequencing of 16S rRNA genes to characterize the gut microbiome of 8 Thoroughbred (Tb) racehorses and study the response to dietary supplementation.

Methods: Faecal samples were collected from 8 Tb racehorses in training. A second sample was collected from all horses six weeks after commencement of twice daily dietary supplementation with an amylase-rich malt extract, designed to enhance pre-caecal digestion. Amplicons for the V1-V3 region of the 16S rDNA gene were multiplexed and sequenced on the 454 resulting in ~25,000 reads per sample. Differences in bacterial community structure were detected by linear discriminant analysis.

Results: Communities were composed of an equal balance between *Bacteroidetes* and *Firmicutes* with a ~5% contribution from other bacterial phyla. The number of observed operational taxonomic units sharing ≥97% nucleotide sequence in our racehorse population was 6000–8000. Horses showed no significant change in the number of OTUs observed after diet supplementation, but significant changes in the relative abundance of both *Bacteroidetes* and *Firmicutes* families were observed.

Conclusions: We demonstrated a highly diverse and complex faecal microbial community in Tb racehorses in the UK. Inter-horse variability is low amongst horses in the same training yard. We identified significant shifts in community profile following dietary supplementation and speculate that these may enhance intestinal health.

Ethical Animal Research: This study was conducted under the University of Liverpool research ethics approval: RETH000363. The informed consent of the trainer was obtained and all samples were obtained non-invasively. **Sources of funding:** private donation. **Competing interests:** none.

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WHERE DO THE COLD BLOOD BREEDS COME FROM AND WHERE DOES POLYSACCHARIDE STORAGE MYOPATHY FIT IN?

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Introduction: Myopathy associated with a gain-of- function mutation in the *GYS1* gene is known in Quarter Horses and cold blood breeds. The mutation occurred between the collapse of the Roman Empire (500 AD) and the upsurge of Carolinian Reign (800 AD). The aim of this review is to reconstruct the development of the cold blood (brachimorph) breeds in Europe using existing literature.

Methods: Literature review

Results: Although archaeological evidence indicates the existence of a mesomorph horse (Cob) type in the early Iron Age, real cold blood breeding only started after 1750. The demand for draught power in the early Industrial Revolution resulted in a large population of cold bloods from 1850 onwards. In Roman times, the huge ugly horses from Thrace were known as "easy keepers" and Ardennes might have been the first land races carrying the *GYS1* mutation. Hunnish horses might have contributed to the prototype cold bloods as well. With mtDNA, Y-chromosome polymorphism, satellite markers and SNP genotyping some indication from where ancestors of particular breeds originate was obtained. Most phylogenetic trees place cold blood breeds, including Fell ponies, Haflinger and Friesian in a clearly different cluster than ponies, saddle horses and Thoroughbreds. Fjords and Mongolian horses are in neighbouring clusters. Since the last 200 years, genetic bottlenecks and the popular sire phenomenon could have caused high *GYS1* H allele frequencies in many continental cold blood breeds.

Conclusions: Cold blood breeds and the *GYS1* mutation likely trace back to the mesomorph tough, easily satisfied horse types from Eastern Europe and central Asia.

Ethical Animal Research: No animals were used in the study. **Sources of funding:** none. **Competing interests:** none.

BIOMECHANICS AND LOCOMOTION

ACCURACY OF GIRTH BASED MEASUREMENTS OF STRIDE CHARACTERISTICS IN HORSES TROTTING ON A TREADMILL

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Introduction: This study aimed to investigate the possibility of obtaining the beginning and end of stance from a girth-mounted Inertial Measurement Unit (IMU), by comparing data from the IMU with data from an opto-electronic system.

Methods: 15 horses were trotted on a treadmill, with markers on hooves and a Girth Marker (GM) on a wireless IMU over the sternum. Proreflex cameras collected 3D marker data. T-tests and Intraclass Correlation Coefficients (ICCs) were used to decipher any difference between diagonally paired limbs; GM and hoof markers; and GM and IMU, the limits of agreement of which were also assessed using a Bland Altman method.

Results: Timings of diagonally-coupled limbs were not significantly different at beginning (P = 0.77 and 0.95), or end of stance (P = 0.60 and 0.46) for right or left diagonal respectively. The diagonal in stance was identifiable from GM y position, with which IMU derived position had 97%, and IMU roll 100% agreement. Stance according to hoof markers did not differ significantly from GM z acceleration at beginning (P = 0.49 ± 0.01, P = 0.92 ± 0.007), or GM z velocity at end (P = 0.84 ± 0.007, P = 0.83 ± 0.007), in right or left diagonal respectively. A strong correlation was seen between beginning and end of stance according to GM and IMU events (each R^2>0.99). Relationships between IMU and GM amplitudes were less convincing, with a significant difference at peak acceleration (P<0.05, Standard Deviation = 2.70 m/s/s, ULOA = 5.93, LLOA = –4.66).

Conclusions: A girth-based IMU can identify temporal stride characteristics in trot on a treadmill, but inferences from amplitude data should be treated with caution until further investigated.

Ethical Animal Research: Approved by Hartpury College Research Ethics Committee. **Sources of funding:** Research self-funded by the first author. **Competing interests:** none.

SKIN DISPLACEMENT IN THE EQUINE NECK

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Introduction: Kinematic studies, using reflective skin markers, are commonly used to investigate equine neck motion in equitation science and for rehabilitation purposes. In order to interpret the registrations accurately, the degree of skin displacement has been described for the limbs and back, but not for the neck. The aim of the present study was to investigate the degree of skin displacement in the equine neck.

Methods: Radiopaque skin markers were applied to the skin over the first six cervical vertebrae of six healthy horses. Radiographs were taken in three standardized neck positions in the sagittal plane: control (horizontal neck), "on the bit" and "nose to carpus". The scales of the images were normalized and calculation of skin displacement was performed by use of a coordinate system, dividing the displacement into measurements on an x-axis parallel to the vertebra's longitudinal axis and a y-axis perpendicular to the x-axis. The non-parametric paired Wilcoxon rank test was employed to study the differences in distances in x- and y-directions, and statistical significance was set to P<0.05.

Results: Between control and "nose to carpus" positions, there were significant differences in the marker locations, related to the underlying vertebra, in x-line for $C_{1–6}$, and in y-line for $C_{3–6}$. The differences in marker locations were between 3 ± 9 and 44 ± 14 mm, depending on the marker.

Conclusions: The outcome of this study indicates that skin displacement is an important factor to consider when investigating equine neck motion with skin marker methodology.

Ethical Animal Research: The study was approved by a local Ethical Committee on Animal Experiments (Uppsala, Sweden), with written consent from the horse owners. **Sources of funding:** none. **Competing interests:** none.

EFFECT OF GAIT AND TURN DIRECTION ON BODY LEAN ANGLE IN THE HORSE

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Introduction: Turning is commonly used as a diagnostic aid in lameness examinations. Systematic movement deviations compared to locomotion

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in a straight line are noted across horses with variation at an individual level. This study aimed to investigate whether deviations from the expected body lean angle, predicted from the ratio of gravitational and centripetal accelerations, are consistent between trot and canter. It was hypothesized that, independent of gait, there would be agreement between the observed and predicted angle with variations on an individual horse level.

Methods: A GPS-aided inertial measurement unit was attached over the sacrum in twenty horses and quantified body lean angle, speed and circle radius in trot and canter on left and right rein. Predicted body lean angle was calculated from speed and circle radius and was compared to the observed body lean (Δobs,pred). A generalized linear model was implemented to study the effect of horse, lungeing direction and gait.

Results: Mean speed and circle radius were 3.3 m/s and 4.9 m in trot and 4.5 m/s and 5.7 m in canter. On average Δobs,pred was –1.1°in trot and –0.8°in canter and was found to be significantly different between horses ($P<0.0001$) and between lungeing directions ($P<0.0001$) but not between gaits ($P = 0.14$). Interactions between horse and direction ($P<0.0001$) and horse and gait ($P = 0.011$) were also found to be significant.

Conclusions: Horses leaned marginally less into the circle than predicted. However, showing significant differences in body lean angle between horses and turn directions but not between gaits, we provide evidence for horse specific adaptations. Whether these are related to laterality or to underlying subclinical lameness needs to be further investigated.

Ethical Animal Research: The study was approved by the local ethics committee. **Sources of funding:** none declared. **Competing interests:** none.

AN INVESTIGATION OF RELATIONSHIPS BETWEEN HORSE AND RIDER PELVIC ASYMMETRY

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Introduction: Asymmetry of the horse and rider partnership is a challenging and complex area. There is limited scientific research regarding the occurrence of misalignments in the horse and rider, and if there is any relationship between the two. This study investigated asymmetry of single horse and rider combinations focusing on pelvis asymmetry.

Methods: 14 single horse/rider combinations (minimum 6 month partnership) on the same daily routine were assessed. Horses and riders were assessed for misalignments of the pelvis, spine, and neck by a fully qualified human and animal (McTimoney) practitioner on the same day. Triplicate measurements of rider dorsal iliac crest (IC) distance (nearest 0.2 cm) and degree of tilt (0d.p) were recorded, sitting and standing, using a PALM palpation meter to calculate IC height discrepancy. Triplicate horse tuber coxae (TC) heights (nearest 0.5 cm) were measured using a plumb line. Difference between means indicated direction and value of asymmetry relative to symmetry of zero. Student's t-test and Pearson's Product Moment correlation examined asymmetry relationships.

Results: Mean rider IC height discrepancy, standing, was 0.89 ± 0.64 cm and sitting, 1.00 ± 0.66 cm. Mean TC height discrepancy was 1.3 ± 1.6 cm. Asymmetry of the pelvis was a feature of 93% of horse/rider combinations, 85% occurring in the same direction. There was a significant positive correlation between horse TC and rider IC height discrepancies ($r^2 = 0.4$, $F = 8.24$, $P = 0.014$).

Conclusions: This study provides positive evidence of a relationship between the direction of horse pelvic rotation and rider pelvic tilt and also between the degree of tilt and rotation. This has implications for physical therapy treatment of horse and rider. Further research of causal effect relationships of the asymmetries is recommended.

Ethical Animal Research: Procedures were reviewed by the McTimoney Chiropractic College Ethical Review Committee. **Sources of funding:** none. **Competing interests:** none.

KINETICS OF THE FORELIMB IN HORSES TROTTING AN UPHILL AND DOWNHILL SLOPE

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Introduction: Equine training often includes running on tracks with different uphill (UP) or downhill (DOWN) slopes and examination of lame horses on a slope can be used to investigate the origin of lameness. However little is known about the kinetics of horses in such circumstances. The aim of this study was to quantify and compare ground reaction force, acceleration and sliding of the hoof in the forelimb of horses trotting an uphill and downhill slope.

Methods: The right front hoof of 5 horses was equipped with a dynamometric horseshoe and an accelerometer allowing the measurement of 3D ground reaction force and hoof acceleration and displacement. The horses were trotted in hand (3.5 m/s) on a 7% inclined asphalt area successively uphill and downhill. Data were analysed using an ANOVA, accounting for correlated repeated measurements within each horse ($P<0.01$).

Results: Speed was not significantly different between UP and DOWN. The maximal longitudinal breaking force of the hoof, maximal vertical force, vertical impulse and longitudinal sliding of the hoof at impact were significantly increased on DOWN compared to UP (respectively +97.7%, +8.4%, +16.6% and +450%). Stride frequency and longitudinal propulsive force were significantly decreased on DOWN compared to UP (respectively –5.1% and –48.7%).

Conclusions: The results of this study (increased longitudinal breaking force and maximal vertical force on a downward slope) are consistent with previous measurements of superficial digital flexor tendon loading on the same area [1]. Conversely increased propulsive force on an uphill slope needs to be taken into account during rehabilitation of horses with deep digital flexor tendon injuries.

Ethical Animal Research: The local Animal Care and Ethics Committee advised that no formal approval was required for this study. **Sources of funding:** Conseil Régional de Basse-Normandie, the Fonds Unique Interministériel, the French Ministry of Agriculture, the FEDER and the Institut Français du Cheval et de l'Equitation (IFCE). **Competing interests:** none.

Reference

[1] Vergari C, Pourcelot P, Ravary-Plumiöen B, Camus M, Holden-Douilly L, Falala S, Chateau H, Crevier-Denoix N. Equine superficial digital flexor tendon force and axial speed of sound: a calibration method under clinical conditions. *Comput Methods Biomech Biomed Engin.* 2012;**15** Suppl 1:12-3.

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INDIVIDUAL LIMB CONTRIBUTIONS TO CENTRIPETAL FORCE GENERATION DURING CIRCULAR TROT

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Introduction: The simplest model of circular locomotion is a point mass moving uniformly on a circle. To produce circular motion, a centripetal force is generated. This study investigated contributions of individual limbs to the net centripetal force in horses trotting on a circle.

Methods: Six horses trotted on a 6 m diameter circle at their preferred speed. Optical motion capture (Motion Analysis Corp) recorded kinematics, while six force plates (AMTI) recorded ground reaction forces (GRF). Whole body center of mass (CoM) was computed using a 23-segment rigid body model. The GRF of each limb was expressed at the CoM in cylindrical coordinates centered at a best-fit circle of the ground path of the CoM. Average vertical, tangential and radial force components were computed for each limb over the whole stride duration. Forces were analyzed using ANOVA with Tukey HSD post-hoc tests.

Results: The maximum ground-path deviation of the CoM from the best-fit circle among all trials was only 2.8% of the radius. The forelimbs contributed an average centripetal (radial) force of 0.49 ± 0.16 N/kg (mean ± SD, normalized to body mass), which was significantly greater ($P<0.001$) than the hind limb contribution of 0.36 ± 0.13 N/kg. There was no significant difference between inside and outside limbs.

Conclusions: The contribution of individual limbs to the centripetal force provided a direct indication of their role in circular locomotion. The forelimbs generated significantly more centripetal force, possibly in direct relation to their greater support of the body mass against gravity.

Ethical Animal Research: The study was approved by the MSU Institutional Animal Care and Use Committee, protocol #06/11-112-00. **Sources of funding:** This study was funded by the McPhail Endowment. SD Starke's collaborative research visit for this project was funded by the British Society for Animal Science, Murray Black Award. **Competing interests:** none.

EFFECT OF SPEED ON STRIDE PARAMETERS AND LIMB LOADING: COMPARISON BETWEEN FORELIMB AND HINDLIMB AT TRAINING TROT ON A FIRM SURFACE

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Introduction: Direct measurement of peak vertical force in equine forelimbs and hindlimbs under training conditions has not been achieved to date. This measurement is possible using dynamometric horseshoes (DHSs). The objectives of this study were to investigate, on a firm surface, the effect of speed on stride parameters and on vertical loading in the forelimb (FL) compared to the hindlimb (HL).

Methods: Right front and hind hooves of 5 trotters were equipped with a DHS. A linear corridor was delimited on a firm sand training track. Each horse alternately repeated trials (harness trot) at 25, 35 and 40 km/h (4 to 6 times at each speed). For each trial, stride parameters and dynamic variables (peak vertical force and impulse, vertical loading rate) were analysed for 10 successive strides. Correlation coefficients were calculated to quantify the association between speed and the studied variables; linear mixed-effects regression models were used to test the significance ($P<0.05$) of these associations.

Results: Stance and stride duration, and their ratio (duty factor) were linearly correlated with speed ($r<-0.82$), both in FL and HL. Peak vertical force was the only variable for which r was higher in HL ($r = 0.80$ vs. 0.50 in FL). The relationship between vertical loading rate and speed had the highest r of all dynamic variables ($r = 0.87$ and 0.83, respectively in FL and HL; $r = -0.75$ and –0.60 for vertical impulse).

Conclusions: The linear relation between peak vertical force and speed demonstrated in the hindlimb differs from previous studies at lower speed. These results stress the necessity of performing biomechanical measurements under real training conditions.

Ethical Animal Research: Formal approval provided by the local Animal Care and Ethics Committee. **Sources of funding:** the Région Basse-Normandie, the FEDER, the Fonds Eperon and the IFCE. **Competing interests:** none.

COMPARISON OF THE LOADING OF LEADING AND TRAILING FORELIMBS IN HORSES AT LANDING

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Introduction: The objective was to compare, using a dynamometric horseshoe (DHS), the longitudinal and vertical components of the reaction force and corresponding impulses in the leading and trailing forelimbs of horses landing after a jump on a competition surface.

Methods: Both forefeet of 3 horses were equipped with a DHS. 1000 Hz films of the right forefoot at landing were synchronized with the DHSs recordings. Within a corridor delimited on a sand arena, each horse jumped a 1.20 m (horse 1) or 1.10 m (horses 2 and 3) high fence while cantering alternatively at both leads. Data were pooled and 37 strides analyzed both for the leading (LF) and trailing (TF) forelimbs. In addition to peak forces and impulses (integral of force over time) in the hoof reference frame, the forces on the right forelimb were projected in the track reference frame to determine true retardatory (TR) and propulsory (TP) phases. Differences between LF and TF were estimated with linear regression models, using generalized estimating equations to adjust for correlated repeated measurements within each horse (for all results, $P<0.0005$).

Results: Maximal vertical force and loading rate were respectively 1.2 and 1.4 times higher on TF. TR-longitudinal impulse was 21 times, and TR-vertical impulse 8 times higher in LF, whereas TP-longitudinal impulse was 4 times, and TP-vertical impulse 2 times higher in TF.

Conclusions: Despite a lower maximal vertical loading, LF plays a major part in the retardatory (load-absorption) phase, while TF is mainly involved in propulsion. Loading of anatomical structures is therefore different in both limbs, which provides insights for interpreting horses' preferred lead at landing depending on which structure is affected.

Ethical Animal Research: The local Animal Care and Ethics Committee advised that no formal approval was required for this study. **Sources of funding:** Région Basse-Normandie, the FEDER, the Fonds Eperon and the IFCE. **Competing interests:** none.

VARIATIONS IN MUSCLE ACTIVITY OF VASTUS LATERALIS AND GASTROCNEMIUS LATERALIS WITH INCREASING DRAFT LOAD

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Introduction: Minimal research has examined the effect of draft loading on equine muscle activity. It is hypothesised that increases in draft load of

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10 and 20% body mass (BM) will increase EMG intensity and duration of vastus lateralis (VL) and gastrocnemius lateralis (GL) during walk.

Methods: Data were collected from six Irish Cob geldings (11 ± 4 years; height: 1.47 ± 0.05 m; BM: 490 ± 65 kg) all trained to draft-load. A modified lorry tyre was used as a loading platform. Surface EMG electrodes were positioned over VL and GL. An accelerometer placed on the left hind hoof collected kinematic data. EMG and accelerometer data at a draft load equivalent to 0 (zero load), 10 and 20% BM were recorded as the horses were led at walk in a 20 m straight line. A stopwatch determined mean speed over 20 m. Mean kinematic and EMG values were determined for 10 consecutive strides. EMG intensities were normalised to each horse. Kolmogorov-Smirnov test explored data normality. Friedman test ($P<0.05$) compared between conditions. A pair-wise Wilcoxon signed ranks test identified where the differences lay.

Results: Data for one horse was excluded because of poor accelerometer data. The remaining horses (n = 5) maintained a constant velocity (1.10 ± 0.02 m/s {SD}) between conditions ($P>0.7$). VL and GL increased their muscular activity in terms of EMG intensity and duration of contraction with increasing draft load from 0 to 10% and 10 to 20% BM ($P = 0.04$; n = 5).

Conclusions: Draft loading can be utilised for strength training following injury or to improve athletic performance.

Ethical Animal Research: The study was approved by the Royal Veterinary College Ethics Committee. **Sources of funding:** Royal Veterinary College. **Competing interests:** none.

EXTERNAL AND RADIOGRAPHIC HOOF ANGLES DIFFER IN THOROUGHBRED FOALS

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Introduction: Poor hoof angle and hoof pastern alignment has been linked to lameness in mature horses. External characteristics, such as the hoof pastern axis and hoof angle, are commonly used to assess dorsopalmar conformation in the same manner in foals. The hoof wall integument and distal phalanx is parallel in healthy mature horses but not in foals: this is not understood.

Methods: The front feet of 22 Thoroughbred foals were radiographed and measured in June and again in November. Lateromedial radiographic images were measured (Osirix) for hoof integument angle and width, and proximal, middle and distal phalanx angles. The data were tabulated in Excel and analysed in Minitab. After assessment for normality (Anderson-Darling), potential relationships were assessed for differences between June and November using paired t-tests and regression analysis.

Results: The dorsal hoof angle became more acute with age, $R^2 = 0.3326$, the mean of June, 59.05 ± 3.662°, was significantly different from November, 53.64 ± 2.7824°, $P<0.0001$. The hoof pastern axes were broken forward in June, 6.1 ± 2.1°, in November 2.5 ± 1.6°, whereas the phalangeal axes were broken back, June,-11.0 ± 7.5°, November, –8.3 ± 4.4°. There was a significant relationship between age and hoof pastern axis alignment, $R^2 = 0.3746$; $P<0.0001$. The phalangeal axis did not correlate with age. The relationship between age and parallelism of the integument was significant $R^2 = 0.4867$; $P<0.0001$.

Conclusions: The cause of the non-alignment of the hoof pastern axis and phalangeal axis in Thoroughbred foals is widening of hoof integument proximodistally and circumferential bone thickening of the distal phalanx. Assumptions of phalangeal angles based on external characteristics cannot be made in foals.

Ethical Animal Research: Approval was given by the University of Central Lancashire Animal Projects Committee. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** World Horse Welfare partially funded this study. **Competing interests:** none.

THE HOOF RENEWAL TIME OF THOROUGHBRED FOALS FROM BIRTH

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Introduction: Thoroughbred foals are born with fully developed hooves and stand within minutes. A ring in the horn occurs at birth and grows down the hoof wall, marking the fetal hoof (distal) and foal hoof (proximal). Birth rings in hoof horn have been noted in other species and used to estimate age. Hoof wall renewal in all ages of horse has been poorly reported; horn growth and complete hoof capsule renewal has not been measured in Thoroughbred foals. Partial hoof wall avulsions are relatively common. The objective was to measure the time taken for the fetal hoof of a newborn foal to grow out and be replaced by hoof grown since birth.

Methods: Thoroughbred foals (N = 150) on four Newmarket stud farms were studied during June to September. The age of the foal and the day that routine hoof trimming removed the remnants of the fetal hoof of the front hooves was recorded. Foals that were on restricted exercise, ill or where the remnants of the fetal hoof still remained after trimming were excluded from the data. 45 foals fulfilled the criteria. The data were tabulated in Excel, analysed in Minitab and assessed for normality (Anderson-Darling).

Results: The mean age at which the fetal hoof grew out was 145 ± 15 days (95% confidence interval, 141.77–147.16).

Conclusions: Thoroughbred foals replace the fetal hoof at twice the speed given for mature horses (270–365 days). Knowing the time of hoof renewal will allow accurate predictions of healing to be made in cases of partial hoof wall avulsion and other hoof lesions.

Ethical Animal Research: Approval was given by the University of Central Lancashire Animal Projects Committee. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** none. **Competing interests:** none.

EFFECT OF X-RAY BEAM ANGLE AND SUPERIMPOSITION ON RADIOGRAPHIC EVALUATION OF KISSING SPINES SYNDROME

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Introduction: Kissing Spines Syndrome (KSS) represents a major challenge in equine practice. Radiographic examination of dorsal spinous processes (DSP) is therefore often included in sale and insurance cases and in diagnosis of back problems. Measurement of interspinous spaces (ISS) on radiographs is often used when evaluating DSPs. Radiographic examination of the equine back usually consists of two to four images causing beam angle differences between the image center and image borders. In addition, radiographs are 2D representations of 3D structures causing superimposition of anatomical structures. The aim of this study was to investigate if x-ray beam angle and superimposition affect radiographic evaluation of ISSs.

Methods: Beam angle effect was investigated using latero-lateral radiographs of the thoracic DSPs. Importance of superimposition of

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anatomical structures was investigated by comparing radiographic measurements with measurements in comparable CT and MR images. Cadaver backs from six horses were included. Four had a diagnosis of KSS, while two were considered normal. Backs were placed in an upright position on a flat surface for all images. Data were analysed using a linear mixed model with a single random factor, ISS. Significance was set at $P<0.05$.

Results: X-ray beam angle significantly influenced measurement of ISS ($P<0.02$) with differences of up to 2 mm, when measured three positions apart. ISS widths measured in radiographs were significantly lower than in comparable CT ($P<0.0001$) and MR ($P<0.0001$) images with differences of up to 3 mm.

Conclusions: These findings suggest that radiographic assessment may result in incorrect evaluation of ISSs.

Ethical Animal Research: Cadaver material was included in the study with signed consent from the horse owners. **Sources of funding:** Access to imaging facilities was kindly donated by the participating equine clinics and Gentofte Hospital (CT). There was no additional funding. **Competing interests:** none.

MYOFASCIAL KINETIC LINES IN HORSES

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Introduction: Myofascial lines are chains of interconnected anatomical structures that functionally direct the basic motion patterns of the musculoskeletal system. They have previously been dissected in humans, where they describe the overall spinal motion of flexion, extension, lateral flexion and axial rotation. They are believed to play a role in functionality, connection, and balance of the body. The aim of this study was to investigate whether similar myofascial lines could be identified in the horse.

Methods: Five myofascial kinetic lines were dissected in 22 horses euthanized for unrelated reasons. The lines were the Superficial Dorsal Line (SDL), Superficial Ventral Line (SVL), Lateral Line (LL), Spiral Line (SL), and Functional Line (FL).

Results: SDL included hind limb flexors and the errector spinae group, which extend the hip and spine. SVL included hind limb extensors, abdominal muscles, and ventral neck muscles, which are structures with antagonistic functions to SDL. LL followed myofascial tissues along the side of the abdomen and produced latero-flexion of the spine. FL and SL formed helical lines concerned with spinal axial rotation. FL connected the front limb to the contralateral hind limb by crossing over the midline at the thoraco-lumbar level dorsal and ventral on the trunk. SL connected one side of the neck with the contralateral front limb and ipsilateral hind limb by a helical course around the body crossing over at the cervico-thoracic and lumbo-sacral junctions.

Conclusions: The SBL, SVL and LL complete a functional anatomical ring, which balances the dorsal, ventral and lateral motion patterns of the horse. The helical FL and SL determine the spinal axial rotation. With minor exceptions all lines mirrored the human lines.

Ethical Animal Research: The study was performed on material collected during post-mortem examination. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** IVCA and The Danish Society for Promotion of Veterinary Science. **Competing interests:** none.

PROBABILITY OF JUMPING SUCCESS OF YOUNG BRAZILIAN SPORT HORSES ASSESSED VIA KINEMATIC ANALYSIS

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Introduction: The study of kinematic patterns of young jumping horses can help to improve selection. This study was carried out to predict free jumping success of young horses using logistic regression to model jumping kinematic variables.

Methods: 108 untrained young Brazilian Sport Horses of both genders were studied. Reflective skin markers were used to identify anatomical reference points. Five free jumps over a vertical obstacle (with a height of 60, 80 and 105 cm for age classes 22–25, 29–32 and 36–39 months, respectively) were recorded. A 100 Hz camera was used, and images were analyzed using Simi Reality Motion Systems®. Successful (n = 1,030) and unsuccessful (n = 200) jumps were analyzed and the following kinematic variables were preselected by principal components analysis and then used as independent variables: last stride length prior to jumping, jumping velocity, take-off distance, withers displacement at jumping, forelimb height at jumping, vertical distance between scapulohumeral and metacarpophalangeal joints, scapulohumeral, radial-metacarpal, tibiometatarsal and withers-fetlock angles.

Results: The effects of take-off distance, forelimb height at jumping and jumping velocity on the jumping result were significant ($P = 0.0091$). An increment of one centimeter in take-off distance and forelimb height led to an increase in the probability of success by 2.6% and 10.6%, respectively. An increase of one meter per second in jumping velocity led to a reduction in the probability of success by 27.8%.

Conclusions: Jumping success is influenced by take-off distance, jumping velocity and forelimb flexibility. Selection of young horses for superior jumping capacity can be aided by kinematic analysis, which may shorten training time and improve performance.

Ethical Animal Research: The research was approved by UFMG Ethics Committee on Animal Research: 267/2008. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do estado de Minas Gerais (FAPEMIG). **Competing interests:** none.

THE EFFECTS OF CHIROPRACTIC TREATMENT ON THE RANGE OF MOTION OF THE CARPUS AND TARSUS OF HORSES

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Introduction: The use of complementary therapies in both humans and animals has grown over the past decade. Equine back problems are more frequently reported as a contributing factor to poor performance, and back dysfunction affects limb kinematics. This study aimed to determine if (McTimoney) chiropractic treatment has an effect on the range of motion (ROM) of the carpus and tarsus.

Methods: Hemispherical 35 mm markers were applied to 7 anatomical landmarks of both forelimbs and hindlimbs of 10 sound, healthy horses from the same riding school with similar workload. The treatment group (n = 5) received (McTimoney) chiropractic treatment for the neck, back, pelvis and front feet. Two digital video recorders set 5.5 m from subjects, filming both sides concurrently, collected 2-D kinematic data at walk and

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trot, 1 day before, and 2 hrs and 7 days after treatment. Data was analysed using Kinovea software, minimum and maximum joint angles were measured and ROM calculated. Statistical analyses included two-way ANOVA, Student's t-test and symmetry indices.

Results: A significant increase in right-side ROM for carpus at walk (+4.0 deg, p = 0.04) and trot (+5.2 deg, p = 0.02) was noted in the treated compared to the non-treated group. For the treatment group, there was a significant change in carpus ROM asymmetry from left towards neutral at walk (P = 0.004) and trot (P = 0.04). Tarsus ROM asymmetry change from left towards neutral was significant (P = 0.02) at trot. No changes were observed in the control group.

Conclusions: These results support the hypothesis that (McTimoney) chiropractic treatment may improve the symmetry of tarsus and carpus ROM of horses. Further research is needed to assess the clinical relevance of these effects.

Ethical Animal Research: Procedures were reviewed by the McTimoney Chiropractic College Ethical Review Committee. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** none. **Competing interests:** none.

SUBJECTIVE EVALUATION OF LAMENESS IN HORSES DURING LUNGEING

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Introduction: Lungeing is an important part of lameness examination since the circular path is thought to accentuate low-grade lameness. Circle dependent, compensatory movement, and pain related asymmetries make lameness evaluation complex. Subjective evaluation, by watching the horse in motion, is standard practice but studies have shown that inter-observer variation is high when assessing lameness (straight line movement). The aim was to estimate inter- and intra-rater agreement between equine practitioners evaluating lameness from videos of sound and lame horses during lungeing.

Methods: Equine practitioners (n = 86), including 43 deemed as experienced, participated in a web-based survey. Horses (n = 23, including two horses with solar-pressure induced lameness) [defined as forelimb-, hind limb-lame or sound from objective kinematic straight line measurements] trotted on a circle in both directions on soft/hard ground surfaces (one condition/video). Of 104 recordings, 60 were selected of which 10 were repeated (numbers chosen to make compliance possible). The practitioners were asked to report which limb was most lame on each video. Kappa statistics were used to analyze the inter-rater and intra-rater agreement. The κ agreement was set to poor <0.3, acceptable 0.31–0.5, good 0.51–0.8, excellent >0.8.

Results: Inter-rater agreement kappa (n = 86) was 0.31 (0.38/0.25 for experienced/less experienced). Evaluating forelimb compared to hind limb lameness agreement increased 11%. Intra-rater agreement kappa (percentiles) was (P10-50-90, 0.21, 0.57, 0.84).

Conclusions: Since identification of the lame limb/limbs is a prerequisite for successful diagnosis, treatment and recovery, the high inter-observer variation when evaluating lameness during lungeing may decrease the accuracy of lameness examinations.

Ethical Animal Research: The experimental procedures were approved by a local Ethical Committee on Animal Experiments (Uppsala, Sweden), and there was a written consent from the horse owners. **Sources of funding:** Swedish-Norwegian Foundation for Equine Research. **Competing interests:** none.

THE EFFECT OF CENTRE OF MASS LOCATION ON PITCHING MOMENTS IN TROTTING HORSES

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Introduction: The diagonal limb support pattern at trot provides pitch and roll stability, controlled by regulating pitching moments about the centre of mass (COM). Correct COM location is critical in calculation of pitching moments, as COM location influences the contribution of loading in the fore and hindlimbs. This study examined the effect of COM location on pitching moments in trotting horses and explored how COM location could influence balance.

Methods: Kinematic (120 Hz) and GRF (4 force plates, 960 Hz) data were collected at trot from three strides of eight horses overground. The position of the COM was determined from the weighted summation of the segmental COMs from previously determined inertial data of Warmblood horses. The COM of the standing model was manipulated cranially and caudally to test the effects of pitching moments during trotting. Pitching moments were calculated for each manipulation of the model and their relationship determined using reduced major axis regression.

Results: Over the stride, the moments must sum to zero to prevent accumulation of rotational motion. This was found when the weight on the forelimbs in standing was 58.7% ± 3% (mean ± 95% C.I.). With this weight distribution moments were typically nose-up changing to nose-down at around 35% of stance, and then reversing again to nose-up in late stance.

Conclusions: Divergence of the COM from the COP creating a vertical force moment arm prior to midstance may aid in accelerating the COM about the hind foot, thereby passively assisting hindlimb propulsion. Lower forelimb weighting resulted in mean nose-up moments. Higher forelimb weighting resulted in mean nose-down moments. Both would cause an accumulation of pitching motion.

Ethical Animal Research: The study was performed with approval from the institutional animal care and use committee under protocol number 02/08-020-00. **Sources of funding:** This study was funded by Michigan State University and the University of Central Lancashire. **Competing interests:** none.

WHAT DO RIDERS WANT FROM AN ARENA SURFACE?

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Introduction: Rider preference appears to be a large factor determining the selection of an arena surface. The negative reaction of the competitors to the surface used for the Olympic test event in 2011 was sufficient to initiate a change in the surface composition used for the Olympic equestrian events in 2012. The aim of this study was to quantify surface characteristics that riders perceive to be the most favoured in a training and competition venue.

Methods: A survey was used to ask riders (n = 342) to describe characteristics that they liked about their favourite venue across Northern England. The two most popular surfaces (both well-established wax, sand and fibre) were harrowed before being measured at several plots. An Orono Biomechanical Surface Tester and traction device were used to compare the functional properties of the two surfaces. The respondent's reasons for choosing their favourite venue were associated with specific surface measures including peak load, rebound timing and traction. A Mann-Whitney U test was used to establish differences between venues.

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Results: The favourite venue was selected because the surface was not too deep or slippery and provided good support, spring and cushioning. The peak load (P = 0.002) and traction (P>0.001) were significantly higher on the favourite surface compared to the second favourite (18.99 kN vs.17.29 kN and 20 Nm vs.19 Nm respectively).

Conclusions: Higher peak load values may be responsible for supporting a good performance and higher traction values could explain why riders felt that the surface was not 'slippery', although this may have negative effects on the horse. The results suggest there is potential to quantify rider preferences.

Ethical Animal Research: The study was approved by an ethics committee at the University of Central Lancashire (reference number: BuSH 057). **Sources of funding:** A student internship was awarded by the University of Central Lancashire. **Competing interests:** none.

EFFECT OF SIDE OF HANDLING ON MOVEMENT SYMMETRY IN HORSES

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Introduction: When horses are trotted in-hand, this is predominantly performed with the handler on the horse's left side. In the context of objective gait analysis, this study investigated whether the side a horse is led from affects movement symmetry measures used to quantify lameness. We hypothesised that the side which horses were handled from would have no effect on head and pelvic movement symmetry.

Methods: Vertical movement of head and pelvis was quantified in 23 horses during trot in-hand with a validated inertial sensor gait analysis system. Established movement symmetry measures commonly used to quantify lameness were calculated for a minimum of 27 strides per condition. Each horse was assessed three times, twice handled from one side, once from the other side, with the initial handling side randomly assigned. Differences in movement symmetry between repeat assessments led from the same side were compared to differences when led from the opposite side using a paired t-test (P<0.05)

Results: Which side a horse was led from had no significant effect on either head (all P>0.754) or pelvic (all P>0.514) movement symmetry. Mean differences for head (and pelvic) movement asymmetry were below 2% or 2 mm (0% or 1 mm) for repeat assessments from the same side and below 4% or 2 mm (0% or 1 mm) for opposite side leads.

Conclusions: The side from which a horse is led does not significantly affect differences in movement symmetry between repeat assessments. Data analysis should be restricted to trials with consistent head carriage and a minimum of 27 strides (previously reported to give a representative estimate of stride-to-stride variability).

Ethical Animal Research: The study was approved by the local ethics committee **Sources of funding:** none declared. **Competing interests:** none.

AN INVESTIGATION INTO THE RELATIONSHIP OF PELVIC MISALIGNMENT WITH FORELIMB HOOF SIZE

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Introduction: The study aim was to determine whether there is a link between pelvic misalignment and uneven forelimb hoof size. Previous research suggests that increased loading increases hoof size, but correlation with pelvic misalignment is unknown.

Methods: 30 healthy, sound, multidiscipline horses, regularly shod/trimmed every 6 weeks, were assessed. Tuber coxae heights were measured in triplicate from the dorsal aspect to ground level, with the horse standing square, using a plumb line and measuring stick. Difference between means indicated value and direction of pelvic misalignment. Using a 300 mm digital vernier calliper, triplicate measurements of hoof width (lateral to medial edge) and hoof length (dorsal to palmar edge) were made of both fore-feet, prior to and after farrier trimming, and were obtained by the farrier (blinded to pelvic measurements) at two shoeing intervals 6 weeks apart. Both methods were tested for reliability and repeatability to ± 1 mm. Statistical analyses included Chi-square, symmetry indices and one-way ANOVA.

Results: Ventral pelvic rotation was related to greater growth in hoof width on the contralateral forelimb and greater growth in hoof length on the ipsilateral forelimb. There was a significant relationship between uneven hoof width and pelvic rotation direction (P = 0.0002), and a significant relationship between uneven hoof length and pelvic rotation directional (P = 6.1 x 10-7). There was a significant relationship between amount of growth in hoof width (P = 0.0002) and hoof length (P = 4.8 x 10-6) with pelvic misalignment.

Conclusions: This study provides evidence of a statistically significant relationship between the alignment of the pelvis and growth of horse's front hooves. Further study would be beneficial in enhancing understanding of the chain of compensatory effects on the equine body.

Ethical Animal Research: Procedures were reviewed by the McTimoney Chiropractic College Ethical Review Committee. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** none. **Competing interests:** none.

PRELIMINARY INVESTIGATIONS INTO PHOTOGRAMMETRIC MEASUREMENT OF HOOF SHAPE

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Introduction: Advances in close-range photogrammetry offer an option for high accuracy measurements of the equine hoof. Automatic camera calibration using coded targets, image matching and triangulation enables 3D model reconstruction from photographs. This study aimed to identify an accurate photogrammetric method to measure proximal hoof circumference (PHC). PHC is currently measured by hand and shown to change in racehorses during training.

Methods: A cadaver hoof was measured by hand, photographed, and laser scanned for method comparison. Then, the left front hooves of two adult horses were photographed under two treatments (exercise and control). Exercise involved 5 minutes brisk trot counter-clockwise on a lunge-line. During both experiments a Canon 40D camera (28–135 mm lens; F5 aperture; 1/60 sec shutter speed) was used. Hooves were marked with permanent marker and coded targets placed on the ground around the hoof for automatic point identification. Photos were taken from 10–16 different locations around the hoof. A paired t-test was used for comparisons and significance set at P<0.05.

Results: Photogrammetry produced a 3D model of the cadaver hoof which was considered good (accuracy of 0.25 pixel RMS). Points on the hoof were manually identified and model creation took 1.5 hours. PHC measurements from photogrammetry differed from the laser scan by 1.25 mm and from hand measurements by 1.73 mm. PHC decreased following exercise (0.8 mm; p = 0.25).

Conclusions: Close-range photogrammetry could be viable for highly accurate and quick measurements of hoof shape. Advantages include; ease of data acquisition, flexibility, low cost, evaluation of the loaded hoof, and high geometric accuracy. To measure only PHC eight images or less could be used. Additional research is required to determine repeatability and accuracy.

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Ethical Animal Research: Procedures were approved by the University of Melbourne's Animal Welfare and Ethics Committee. **Sources of funding:** University of Melbourne. **Competing interests:** none.

TRACK SURFACE IDENTIFICATION USING AN INSTRUMENTED HORSESHOE

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Introduction: Track conditions in Thoroughbred racing significantly impact performance and injury. However, surface effects on limb biomechanics at racing speeds are poorly understood.

Methods: A racing shoe was modified to contain inertial transducers and a strain gauge to provide motion kinematics and a measure of hoof/surface interaction load. A two-phase non-linear surface reaction model was defined to represent the hoof deceleration phase, and the hoof penetration phase where the surface reaction force was represented by a quartic stiffness profile and a fixed damping term. Model parameters were determined using an "output error" iteration method for 25 strides at medium gallop. Parameters were compared using a standard t-test.

Results: The sand track had a significantly greater ($P<0.0001$) maximum penetration of 61 ± 10 mm (mean ± SD), than the grass track (41 ± 0.5 mm). Both stiffness parameters were significantly different between the two tracks ($P<0.0001$), but damping parameters did not differ significantly. Hoof impact at high speed involved decelerations of over 100 'g' occurring within milliseconds.

Conclusions: This study demonstrated that a surface reaction model using data acquired from an instrumented horseshoe, distinguished between surface reaction profiles of a sand track and a grass track, as a step towards improving track rating methods. A further challenge will be to develop independent checks on instrument calibrations.

Ethical Animal Research: The study was approved by the University of Melbourne Animal Ethics Committee. **Sources of funding:** This study was funded by the Rural Industries Research and Development Corporation under project PRJ-006479, and by a University of Melbourne Research Collaboration Grant. **Competing interests:** Colin Martin is the Director of SPERERO Pty. Ltd. a consulting and innovations company.

COMPARISON BETWEEN INERTIAL SENSORS AND MOTION CAPTURE SYSTEM TO QUANTIFY FLEXION-EXTENSION MOTION IN THE BACK OF A HORSE

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Introduction: The development of miniaturized inertial sensor technology (IMU) opens new possibilities to assess equine back motion in real-life situations. The consistency of IMU for measuring dorsoventral displacement of the back has been assessed but orientation has not. The aim of this study was to assess accuracy and consistency of miniaturized inertial sensors for quantification of flexion-extension of the equine back, compared to motion capture (mocap) as a gold standard.

Methods: One sound horse was trotted (3.5 m/s) and cantered (9 m/s) on a treadmill with synchronized mocap and IMU data collection. The amplitudes of two flexion-extension angles were calculated between 3 IMUs (T12-T16-L2) and with a triad of markers linked to each IMU during 15 consecutive strides for two recordings at each gait. Consistency of sensor measurements was assessed using Lin correlation coefficient.

Results: Thoracic and thoracolumbar angles showed respectively a Lin correlation coefficient of 0.96 (excellent) and 0.87 (good). The difference (mean ± SD) between IMU and mocap were, respectively for the thoracic and thoracolumbar angles, 0.57 ± 0.44 and 0.65 ± 0.47 degrees during trotting, and 0.83 ± 0.67 and 0.63 ± 0.44 degrees during canter.

Conclusions: Angle values calculated with IMU data showed acceptable accuracy consistency for quantification of flexion-extension movement in a horse's back. These results are encouraging for future development of the method in real-life situations where mocap systems are unsuited for the quantification of back movements.

Ethical Animal Research: None declared. **Sources of funding:** The authors thank the Institut Français du Cheval et de l'Equitation, the Association Nationale de la Recherche et de la Technologie and the Conseil Régional d'Aquitaine for financial support, the Pôle Hippolia for logistical support, and CIRALE for loaning the treadmill and the horse. **Competing interests:** none.

THE POINT OF APPLICATION OF THE GROUND REACTION FORCE MOVES IN CIRCLING HORSES

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Introduction: The position and magnitude of the ground reaction force (GRF) relative to the hoof influences loading within the limbs. In this study, we investigated changes to the point of force application (PFA) of the GRF in horses walking and trotting on circles compared to a straight line.

Methods: 8 horses were lunged in 6 m diameter circles (left and right), and led straight, at walk and trot over force plates on firm ground. Hoof-mounted retro-reflective markers were tracked using a motion capture system (Motion Analysis Corp). The PFA was computed in a hoof-local coordinate system during 2018 strides. The instantaneous PFA was weighted by the GRF magnitude to compute an average PFA position within the hoof print for each total stance phase. The average PFAs were analyzed using an ANOVA in which type of locomotion (circle or straight), gait (walk or trot) and hoof (fore or hind) were used as independent variables. A Tukey post-hoc analysis identified pair-wise differences.

Results: At trot, the PFA moved toward the center of the circle by 19.8 ± 10 mm (mean ± SD, $P<0.001$). At walk there was no significant difference in PFA between straight and circle. The hoof (fore or hind) did not significantly affect the PFA shift.

Conclusions: The significant medio-lateral shift of PFA suggests that the balance of limb loading changes during circular locomotion. This is likely to affect the medio-lateral balance in the distal joints and cause sub-optimal loading of limb structures.

Ethical Animal Research: The study was approved by the MSU Institutional Animal Care and Use Committee, protocol #06/11-112-00. **Sources of funding:** This study was funded by the McPhail Endowment. SD Starke's collaborative research visit for this project was funded by the British Society for Animal Science, Murray Black Award. **Competing interests:** none.

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A COMPARISON OF PIAFFE IN COMPETITION WITH THE FEI DEFINITION OF THE MOVEMENT

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Introduction: Requirements of competitive dressage should be reviewed periodically to inform training of horses and judges. The aim of this study was to determine whether features of the FEI definition of piaffe are achieved in competition and to correlate the judges' scores with the degree of conformity to the definition.

Methods: Videography (60 Hz) of 3 piaffe strides (performed at marker D within a field of view 6 x 4 m) from 60 Grand Prix horses was used to measure croup height (CH), poll height (PH), the highest point of the neck (PN), peak height of the fore (FTpeak) and hindlimb toe (HTpeak), the level of a point half way down the cannon (MC) and the level of the fetlock joint (FJ) to within ± 0.01 m. Paired t-tests were used to compare CH in piaffe with that in halt, PH with PN, FTpeak with MC and HTpeak with FJ during piaffe to determine if the criteria within the definition were met. Piaffe scores from 5 judges were obtained from a subset of 17 horses.

Results: CH was higher in piaffe than in halt ($P<0.001$). PN was higher than PH in piaffe ($P = 0.001$). FTpeak was lower than MC ($P = 0.001$) and HTpeak was lower than FJ ($P = 0.001$). There was no significant difference ($P = 0.836$) between the scores awarded to each horse but also no correlation between the scores and PH ($P = 0.331$), CH ($P = 0.829$), FTpeak ($P = 0.784$) or HTpeak ($P = 0.924$).

Conclusions: Piaffe performed in competition does not conform to the FEI definition of the movement although judges' scores appear consistent. This finding suggests that the definition of the movement or judge training should be reviewed.

Ethical Animal Research: Project approved by Hartpury College Research Ethics Committee. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Hartpury College. **Competing interests:** none.

EFFECT OF AN ORAL JOINT SUPPLEMENT ON ORTHOPAEDIC EVALUATION SCORES AND LIMB KINEMATICS

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Introduction: Numerous oral joint supplements are available, but there is limited research into their efficacy. This study evaluated the effect of an oral joint supplement on orthopaedic evaluation scores and limb kinematics in horses.

Methods: 24 mature horses (71% 1–4/10 lame) were included. Exclusions were poor body condition, health problems, or lameness >4/10. Supplement S (FlexAbilityTM, Science Supplements, containing chondroitin sulphate 162 g/kg, glucosamine 190 g/kg, vitamin C 80 g/kg, methyl sulphonyl methane 256 g/kg, DHA 66 g/kg); EPA 34 g/kg; or placebo P (carrier/flavours only) were fed to horses for 21 days each in a random order triple-blind crossover design. Horses were evaluated at day 0 (baseline), 21 (after first treatment) and 42 (after second treatment). Clinical orthopaedic evaluation for straight line and lungeing circle (walk and trot), and during ridden exercise (walk, trot and canter) was performed. Side-view high-speed motion-capture (240 Hz) determined midstance tarsal flexion, carpal extension and fetlock extension for straight line trotting. All evaluators were blinded to treatment. Effect of treatment, sequence, limb and interactions were investigated using linear mixed models.

Results: S was associated with significantly lower lameness grade in a straight line ($P = 0.001$) with individual horses improving up to 2/10 grades and circle ($P = 0.010$) than either P or baseline. For horses with hindlimb lameness, supplement S was associated with significantly greater tarsal flexion than baseline (4.2% greater, $P<0.02$) or P (2.7% greater, $P<0.037$).

Conclusions: Oral administration of supplement S was associated with less lameness than P or baseline. Increased midstance tarsal flexion of lame limbs may indicate improved mobility or comfort during peak loading.

Ethical Animal Research: Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** World Horse Welfare, Science Supplements. **Competing interests:** Rebecca Frost is employed by Science Supplements. Vicki Adams and Rachel Murray are involved with Science Supplements on a consultancy basis.

EFFECTS OF THORACIC POSTURE ON THE NUCHAL LIGAMENT IN FOETAL FOALS

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Introduction: Controversy surrounds certain head and neck postures in sport horses and their potential impact on the nuchal ligament.

Methods: Three dead near-term equine foetuses in lateral recumbency with upper-side neck musculature removed to expose the nuchal ligament were placed in 6 head and neck positions (HNP's) between extreme hyperflexion and hyperextension, along with 3 thoracic positions: Low, Medium and High. In each of these 18 positions, the funicular length, lamella dorsoventral width at the level of C4, and craniocaudal length of the 5 major lamella bands of the nuchal ligament were measured manually.

Results: In intermediate HNP's (HNP1, HNP2), thoracic postures significantly impacted nuchal ligament measurements. Compared to the Medium thorax position, depression and elevation of the thorax decreased lamella sheet widths by 24% ($P = 0.014$) and 11% ($P = 0.267$) respectively for HNP1, and by 8% ($P = 0.01$ for both depression and elevation) for HNP2. More extreme head and neck positions were not associated with any change in measurements of the nuchal ligament with changes in thoracic position.

Conclusions: A decreased width suggests increased tension in an elastic structure. Hence, if the anatomical connections are similar in adults, extreme head and neck postures may reduce the effect that thoracic posture might have on the nuchal ligament in certain unnatural positions required in sport horses and may interfere with normal elastic energy storage in the nuchal ligament during movement.

Ethical Animal Research: The study was performed on material collected during post-mortem examination. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** none. **Competing interests:** none.

STRIDE RATES OF QUARTER HORSES AND THOROUGHBREDS DURING RACES OF SHORT AND CLASSIC RACE DISTANCES

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Introduction: Racing Quarter Horses (QH) typically compete at shorter distances and faster speeds than Thoroughbreds (Tbred). The two components of speed are stride rate and stride length. The hypothesis of

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this study is that racing QH have greater stride rates than Tbreds, and that stride rate decreases as distance increases, even within breed.

Methods: Five runnings of sprint and classic distance stakes races for both QH (Texas Twister Stakes, 100.6 m; Champion of Champions, 402.3 m) and Tbred (Breeders' Cup Sprint, 1,207.0 m; Breeders' Cup Classic, 2,011.7 m) contested from 2008 to 2012 were obtained and viewed by five individuals. Individuals watched each race three times (in slow motion when needed) and the average number of strides per second taken by the winning horse was calculated. Using the PROC Mixed Procedure of SAS, the averages from the five individuals were used to compare differences between breeds, and between distances within a breed. The stride rates were also used to determine average stride length by factoring in length of each race.

Results: The average stride rate was greater for QH than for Tbred (2.88 vs 2.34 strides/sec; $P<0.0001$). Additionally, stride rate decreased ($P<0.0001$) as distance increased (2.96, 2.81, 2.45, 2.23 strides/sec, respectively). Stride length was longest in the Tbred races and shortest in the 100.6 m QH race ($P<0.0001$).

Conclusions: The Tbred stride rates reported here, and associated respiratory rates, have been previously reported and are remarkable. However, QH rates are nearly 25% greater. The associated rapid acceleration and deacceleration of the lower limb and quick breathing rate may have implications for skeletal and respiratory soundness.

Ethical Animal Research: No animals were used in this study and all information was obtained from previously conducted races for which video was publicly available. **Sources of funding:** Michigan State University and MSU AgBioResearch provided funding for support of investigators conducting this study. **Competing interests:** none.

DEVELOPMENT OF THE EQUESTRIAN ARENA SURFACES FOR THE 2012 OLYMPIC GAMES

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Introduction: The equestrian arenas at the 2012 Olympic Games were constructed on a unique raised platform. Arena surfaces can be implicated in risk of injury and affect performance, therefore functional properties were examined to ensure preparation was appropriate for this unusual construction. The objective of this work was to assess surface hardness, load carrying capacity and uniformity during the development of a suitable surface for the 2012 Olympic Games. Data were compared from the Olympic site at a test event in 2011, pre-London 2012 and from developmental test arenas on and off a raised platform.

Methods: *In-situ* systematic sampling was used across arenas. A biomechanical surface tester quantified load carrying capacity (peak load), a Clegg impact hammer was used to assess hardness (peak deceleration) and moisture data were analysed in the laboratory. Contour maps and GLMs (applying the arena's structural features as explanatory variables where appropriate) were used to highlight surface heterogeneity.

Results: Significant variation in peak deceleration across the surface was detected at the test event in 2011 ($P<0.0001$). Peak load ($P<0.0001$) and peak deceleration ($P<0.0001$) were higher pre-London 2012, partly due to enhanced stiffness of the base layer. Higher moisture content found pre-London 2012 ($P<0.001$) was considered beneficial for surface consolidation. There was no significant difference between data collected on or off a raised platform following the developmental work. Results highlight the importance of effective water management and sub-surface construction.

Conclusions: Findings from this work guided decisions made by the surface providers for the 2012 Olympic Games and advanced understanding of methods most appropriate for assessing arenas *in-situ*.

Ethical Animal Research: This study was approved by the Faculty of Science and Technology ethics committee at Anglia Ruskin University, Cambridge, UK. **Sources of funding:** The project was conducted as an independent assessment and consultancy for the surface providers Andrews Bowen Ltd, at Greenwich Park for London 2012. Submission of the work has been approved by Andrews Bowen Ltd. **Competing interests:** none.

PRESSURE PLATE EVALUATION OF FORELIMB TOE-HEEL AND MEDIO-LATERAL HOOF BALANCE ON A HARD VERSUS A SOFT SURFACE IN SOUND PONIES AT THE WALK AND TROT

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Introduction: Pressure plate analysis has been limited to measurements on a hard surface, whereas horses routinely perform on a soft substrate.

Methods: Five sound unshod ponies were walked and trotted over a pressure plate under 2 different conditions: first, the plate was covered with a 5-mm rubber mat, and then with an additional 50-mm layer of sand/synthetic fibres. Peak vertical force (PVF), vertical impulse (VI), peak vertical pressure (PVP), contact area (CA) and stance duration (ST) were obtained. Toe-heel and medio-lateral hoof balance curves of the vertical force were calculated (126 Hz). Hard vs. soft surfaces were compared using ANOVA and Kruskall-Wallis test with pairwise comparisons.

Results: PVF, VI, PVP and ST decreased on the soft substrate, while CA increased ($P<0.001$). At impact, there was more even load distribution between the toe and heel region on the soft vs. hard surface, both at walk ($P<0.001$) and trot ($P = 0.039$), and between the lateral and medial zone at walk ($P = 0.006$), but not at trot. At mid-stance, there was more loading of the toe region on the soft vs. hard surface at walk ($P = 0.044$) and at trot ($P = 0.005$), while the medial and lateral zone were loaded equally.

Conclusions: The differences observed may be due to dampening effects, measurement errors or gait adaptations. Due to the lack of simultaneous force plate data this cannot be definitively identified, but the first option seems most likely. This study opens perspectives for evaluation of the effects of conformational deficits and therapeutic farriery on hoof loading in relation to arena composition.

Ethical Animal Research: All ponies in the study are part of the faculty teaching herd, institutional guidelines were followed. **Sources of funding:** Ghent University. **Competing interests:** none.

THE EFFECT OF TRAINING ON STRIDE PARAMETERS IN A COHORT OF 40 TWO YEAR OLD RACEHORSES

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Introduction: The biomechanical effects of training on gait in the Thoroughbred are not fully understood. We aimed to assess the effect of training, surface and incline on stride parameters.

Methods: 40 two-year-old Thoroughbred racehorses entering training were recruited and followed from December 2012 until September 2013. Gait analysis was performed at eleven time points by equipping each horse

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with an inertial measurement unit (IMU) with inbuilt GPS, mounted on the dorsum immediately caudal to the most caudal palpable lumbar dorsal spinous process. Horses were exercised as per their regular training regimen on a variety of training surfaces and inclines. Data were analysed using custom software written in Matlab (Mathworks, UK), and statistical analysis was performed using R (R Foundation for Statistical Computing, Austria). A linear mixed model was used, with horse as a random effect and weeks in training, training surface, curve and incline as fixed effects.

Results: A total of 85,497 strides at speeds of between 6.00 and 19.03 ms^{-1} were analysed. Stride length increased by 0.33 m ± 4.3 x 10^4 with each 1 ms^{-1} increase in velocity. Training causes a reduction in stride length of 0.004 ± 0.0001 m per week and an increase in stride frequency of 1.29 x 10^3 ± 4.1 x 10^5 per week. Shallow inclines, steep inclines and declines cause a decrease in stride length of 0.07 ± 0.003 m, 0.10 ± 0.005 m and 0.04 ± 0.004 m respectively. Turf and peat moss training surfaces caused an increase in stride length of 0.03 ± 0.004 m and 0.06 ± 0.007 m respectively.

Conclusions: This study provides an insight into the effects of external factors on the gait of young Thoroughbreds in training. Ongoing research will further investigate these effects and interactions.

Ethical Animal Research: The study was approved by the local ethics committee. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Horserace Betting Levy Board. **Competing interests:** none.

EFFECT OF PELVIC TILT ON SYNCHRONISATION AND STABILITY OF RIDERS IN SITTING TROT

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Introduction: Rider movement may influence horse-rider interaction, yet little is known about what physical attributes affect postural stability. This study aimed to compare pelvic control and postural stability of riders on the floor and whilst riding.

Methods: 33 riders were assessed by a physiotherapist and subjectively graded according to ability to tilt the pelvis in isolation: 1 = poor, 2 = better plus compensations, which were either A = lumbar spine inclusion B = full body lean, 3 = good. Riders wore markers on (1) greater trochanter of the femur, (2) iliac crest, (3) greater tubercle of the humerus. A marker was placed over the horse's tuber sacrale (4). Two-dimensional high-speed motion-capture (240 Hz) recorded four strides of collected sitting trot in a straight line from the left side. Distance between markers was measured at midstance, maximum hindlimb protraction and retraction. Synchronisation was determined by the difference in time (secs) between the tuber sacrale of the horse and pelvis of the rider at these stride points. One-way ANOVA was used to compare synchronisation between groups. Student's independent t-test was used to compare marker distance.

Results: For pelvic tilt, no grade 3 riders were seen. Grade 2 riders were more synchronised with the horse during left hind diagonal suspension phase ($P = 0.011$) and had smaller horizontal distances between markers 1–2, 2–3 and 1–3 respectively at all points of the stride (midstance: $P<0.042$; hindlimb protraction: $P<0.049$; hindlimb retraction: $P<0.013$). Comparing compensation types, grade A riders were better synchronised with the horse at midstance phase ($P = 0.014$).

Conclusions: Results suggest that control of pelvic movement may have an impact on horse-rider synchronisation.

Ethical Animal Research: This project was approved by the Animal Health Trust Clinical Research Ethics Committee. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** The Elise Pilkington Charitable Trust, British Dressage and Dr. Bechtolsheimer. **Competing interests:** none.

OPTIMAL GAIT PARAMETERS FOR QUANTIFYING THE EFFECT OF DIAGNOSTIC ANALGESIA IN HORSES

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Introduction: Evaluating responses of horses to diagnostic analgesia is subject to bias. Pelvic movement symmetry can be measured accurately with inertial sensors, but it is unclear which of the many symmetry measures best reflect changes. It was hypothesised that objective parameters mimicking commonly observed visual parameters used by veterinarians (difference between upward/downward sacral movement and between amplitudes of left and right tuber coxa motion) would show the largest, most consistent responses.

Methods: 13 horses underwent visual lameness scoring and independent gait assessment with inertial sensors during clinical lameness investigations. Multiple movement symmetry parameters were calculated and changes before/after diagnostic analgesia regressed against change in lameness grade. Confidence intervals of slopes of regression lines were assessed to identify significant changes.

Results: All parameters showed variation between horses and across lameness grades. Three measures documented a consistent increase in symmetry for each grade of change in lameness score: difference between sacral displacement minima, and upward movement difference and range of motion difference between the tubera coxae. A change in symmetry of 9–13% for each grade of increase in lameness can be expected.

Conclusions: The three most promising pelvic parameters for consistent quantification of changes due to diagnostic analgesia in the hindlimb of lame horses were identified. Difference in upward movement between left and right tuber coxae is the most sensitive but requires simultaneously focusing on pelvic and limb movements and may therefore be more difficult to assess visually. Showing a similar response to diagnostic analgesia, the difference in movement amplitude between left and right tuber coxae may be more easily perceived since it does not require simultaneous focus on limb movement.

Ethical Animal Research: The study was approved by the local ethics committee. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** None declared. **Competing interests:** none.

THE EFFECT OF NOSEBAND TIGHTNESS ON TENSILE FORCES WHILE USING SIDE REINS ON HORSES

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Introduction: The fixation of the bridle, particularly the noseband, is important in the horse-rider interaction. The goal of this study was to show the influence of noseband tightness on tensile forces elicited in side reins under traction to position the horse's head.

Methods: Ten Warmblood horses (2 mares, 8 geldings; mean age: 12.4 ± 5.3 years) were assessed during walk and trot on a treadmill. Side reins were used to position the horse's head so that the nose was nearly vertical. Measurements were made with a correctly tightened noseband (two fingers space between the noseband and nasal bone = N1) and with a

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tight noseband (= N2). Rein forces were measured with an electronic sensor based on a force sensing resistor (27 g, sample rate 120 Hz, Interlink Electronics corp.), synchronized with a kinematic system (10 cameras, 120 Hz, Motion Analysis corp.). The force sensors were calibrated with masses of 1 Kg (=9.81 N) and 4 Kg (=39.24 N). Statistical analysis was performed using the software SPSS. Normality of data was checked with the Kolmogorov-Smirnov-test and data were analysed with repeated measures ANOVA (P<0.05).

Results: Side rein forces in N1 were significantly higher than in N2 during walk and trot. Maximum forces at walk were 8.6 ± 0.9 N for N1 and 6.7 ± 0.95 N for N2. At trot the forces (7.2 ± 0.95 N for N1; 5.96 ± 0.9 N N2) were significantly reduced compared to walk. There was no significant difference between left and right rein forces.

Conclusions: This study demonstrated that noseband tightness has an influence on rein tension. The rein forces that were exerted were lower than in ridden horses.

Ethical Animal Research: This study was approved by the local ethics commission. **Sources of funding:** University of Veterinary Medicine of Vienna. **Competing interests:** none.

THE EFFECT OF TWO DIFFERENT BITS ON TENSILE FORCES IN SIDE REINS

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Introduction: The effect of bits on the mouth of the horse is a source of controversy in animal welfare discussions. The goal of this study was to determine the influence of two different bits on tensile forces in side reins applied to exercising horses.

Methods: Ten Warmblood horses (2 mares, 8 geldings; mean age: 12.4 ± 5.3 years) were assessed during walk and trot on a treadmill. Side reins were used to position the horse's head so that the nose was nearly vertical. Two different bits were utilised: a double jointed snaffle (bit 1) and a mullen mouth snaffle (bit 2). Rein forces were measured with an electronic sensor based on a force sensing resistor (27 g, sample rate 120 Hz, Interlink Electronics corp.), synchronized with a kinematic system (10 cameras, 120 Hz, Motion Analysis corp.). The force sensors were calibrated with masses of 1 Kg (=9.81 N) and 4 Kg (=39.24 N). Statistical analysis was performed using the software SPSS. Normality of data was checked with the Kolmogorov-Smirnov-test and data were analysed with repeated measures ANOVA (P<0.05).

Results: Maximum tensile forces differed significantly between the two bits. Maximum forces at walk were 16 ± 1.9 N using bit 1 and 15.7 ± 1.9 N using bit 2. At trot the forces (12.7 ± 2.0 N bit 1; 10.4 ± 1.8 N bit 2) were significantly reduced compared to walk. There was no significant difference between left and right rein forces.

Conclusions: The type of bit has an effect on the tensile forces in the side reins and hence on the forces exerted on the mouth of the horse.

Ethical Animal Research: This study was approved by the local ethics commission. **Sources of funding:** University of Veterinary Medicine of Vienna. **Competing interests:** none.

CAN LOCOMOTION ASYMMETRY IN YOUNG TROTTERS PREDICT EARLY RACE PERFORMANCE?

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Introduction: Lameness is the most common health problem of racehorses and may affect both welfare and performance. The aim of this study was to investigate if locomotion asymmetry in a group of young horses was related to delayed qualification for races.

Methods: Locomotion asymmetry was measured every 1–3 months with a wireless inertial sensor based lameness evaluation system in 16 horses fourteen times between September of the year they were 1-year olds and May of the year they were 3-year olds. As 3-year-olds they passed their qualification race and after this, two subgroups were created for the study based on whether they passed their qualification race before June 21st (early group, n = 6) or after September 19th (late group, n = 6). The groups were compared with respect to the differences in vertical head (forelimbs) and pelvis (hindlimbs) movement (as measured by the Lameness Locator, Equinosis) during each stride cycle at trot. The vector sums (VS) of maximum and minimum difference from each sensor were calculated and used for statistical analyses. ANOVA (Proc mixed model including individual {random}, date and group) was used to analyse differences between groups.

Results: The late group had higher VS for forelimbs (16.0 ± 1.0) than the early group (12.6 ± 1.0) (P = 0.04) in total over the whole period. The VS for hind limbs did not differ between groups.

Conclusions: Front limb locomotion asymmetry in young horses in training is associated with delayed qualification for races. However, further studies are warranted to investigate causality.

Ethical Animal Research: The study was approved by the local ethics committee in Umeå, Sweden. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Swedish University of Agricultural Sciences, the Swedish National Trotting School, Wången, the Swedish Trotting Association, The Swedish Horse Council Foundation, the Swedish Horse Racing Totalisator Board (ATG), Trioplast AB and Dow Chemicals. **Competing interests:** none.

CHANGES IN KINEMATICS DURING REPEATED JUMPING

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Introduction: Kinematic studies allow the assessment of variations in body position during jumping. This study evaluated changes in kinematic variables in horses during a jumping test.

Methods: Eight horses equipped with reflective skin markers were submitted to a jumping test consisting of 70 jumps in 4 series of 10, 15, 20 and 25 times each over a 1.10 m vertical obstacle. Six jumps per series were recorded; a heart rate of ≤ 90 bpm was the criterion to begin the next series. A 25 Hz calibrated camera was placed 10 m from the fence, and 2D images were analyzed. The variables were: overtracking at jumping; take-off and landing distances; jump length; distance between lowest point of the fore and hind limb and the fence; height of head, withers and croup in relation to the ground when passing the fence; and maximum withers height at jumping. Data were analysed with an ANOVA with a post-hoc Tukey test (P<0.05).

Results: There was a reduction in take-off distance (1.65 m to 1.46 m, P = 0.03), landing distance (1.99 m to 1.71 m, P = 0.001), jump length (4.51 m to 4.11 m, P = 0.005), left forelimb distance height above the fence (9.4 cm to 5.1 cm, P = 0.03) and maximum withers height at jumping (2.0 m to 1.95 m, P = 0.001) from the first to the third series. Overtracking before jumping increased from 32 cm to 48 cm (P = 0.003) and there was a reduction of right forelimb height above the fence from 7.3 cm to 2.9 cm (P = 0.04), and of withers and croup height above the fence from 2.0 m to 1.95 m (P = 0.001) and 1.93 m to 1.86 m (P<0.0001) respectively from the first to fourth session.

Conclusions: The increase in the number of jumps resulted in a decrease of take-off and landing distances, and in the distance between forelimb and fence and the withers and croup height, all indicating decreased jumping efficiency.

Ethical Animal Research: The research was approved by UFRRJ Ethics Committee on Animal Research: UFRRJ/ COMEP-UFRRJ: 263/2012.

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Sources of funding: CNPq, CAPES and FAPERJ. **Competing interests:** none.

THE EQUINE CERVICAL SPINE: COMPARING MRI AND CONTRAST-ENHANCED CT IMAGES WITH ANATOMIC SLICES IN THE SAGITTAL, DORSAL AND TRANSVERSE PLANE

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Introduction: The impact of cervical pathology on performance is of great importance to the equine industry. Accurate diagnosis of cervical disease with imaging modalities, including CT and MRI, requires thorough appreciation of normal cervical anatomy. The objectives of this study were to describe in detail the 3-dimensional anatomy of the equine cervical spine by comparing anatomical sections with corresponding MR and contrast-enhanced CT images in the sagittal, dorsal, and transverse plane and to determine the ability of MR and contrast-enhanced CT imaging to visualize anatomical structures in the cervical spine.

Methods: Three cervical spines of young adults (3–8 years), were collected immediately after humane euthanasia. The spine was stabilized on a frame in a natural flexed position with an angle of 20-degrees. MR and contrast-enhanced CT imaging was performed within 6 hours after euthanasia. Anatomical sections of 1 cm were made in the sagittal, dorsal, and transverse plane and compared with corresponding CT and MR images. The intervertebral disk thickness, facet joint angle, amount of cerebrospinal fluid and amount of synovial fluid within each facet joint were quantified.

Results: The anatomical locations of clinically important structures including the facet joints, spinal cord, cervical nerve roots and intervertebral disks were reliably identified in the anatomical sections and their corresponding MR images. Contrast-enhanced CT images depicted all osseous borders; MR images were superior for soft tissue structures.

Conclusions: This study enhances the understanding of normal cervical spine anatomy and the diagnostic usefulness of cervical MRI and contrast-enhanced CT in the horse.

Ethical animal research: This study was approved by the animal care and use committee of the Mississippi State University, MS, USA. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Office of Graduate Research and the Department of Clinical Sciences of the College of Veterinary Medicine, Mississippi State University. **Competing interests:** none.

TOWARDS RELIABLE OBJECTIVE LAMENESS QUANTIFICATION ON THE CIRCLE

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Introduction: Lungeing is part of the standard lameness examination, however it systematically affects movement symmetry even in sound horses. We evaluated a method for objective lameness detection on the circle accounting for this asymmetry bias.

Methods: Twenty-six horses undergoing lameness examination were equipped with inertial sensors (Xsens) on head, sacrum and tubera coxae. Asymmetry in vertical displacement was quantified for trot on straight line and circle using multiple measures. Horses were classified lame when falling outside the directional reference range of sound horses (directional = 'normal' margins and lame limb depending on rein and sign of measure). Objective classification on the circle was compared to subjective evaluation, final diagnoses and objective classification on the straight calculating the proportion of matches. Regression analysis evaluated the association of change in asymmetry between straight line and circle and baseline asymmetry on the straight.

Results: The match between objective classification on the circle and the exact fore-/hindlimb(s) scored lame subjectively was 81% / 79%. The match with the diagnosed limb(s) was 71% / 85%. There was a high frequency of false positives compared to subjective evaluation. Objective evaluation on the circle added new information compared to assessment on the straight line in 70% (forelimb) / 58% (hindlimb) of instances (contralateral limb lame, bilateral lameness, lame on circle but sound on straight). Change in asymmetry was not associated with baseline lameness ($P \geq 0.062$).

Conclusions: Objective lameness evaluation on the circle makes a valuable contribution to patient assessment: e.g. subtle lameness falling within the margins of 'normal' on the straight or bilateral lameness could be identified. In future, the predictive value of the proposed technique should be determined.

Ethical Animal Research: The study was approved by the local ethics committee. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Sandra Starke's PhD studentship was funded through the Mellon Trust via the Royal Veterinary College. **Competing interests:** none.

IS FOOT PLACEMENT RELATED TO BODY MOVEMENT ASYMMETRY DURING CIRCULAR LOCOMOTION?

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Introduction: The mechanics of circular locomotion are relevant to equine training and lameness evaluation. Circular trotting is associated with asymmetries in upper body movement. This study investigated whether changes in foot placement explain upper body asymmetry and whether this asymmetry is dependent on centripetal force.

Methods: Six functionally sound horses walked and trotted on a 6 m diameter circle and straight line at their preferred speed. Optical motion capture (Motion Analysis Corp.) recorded foot position in 3D space. For each limb, horizontal foot placement was calculated in a body-based reference system. Vertical displacement asymmetry (Vector Sum, VS) was calculated from inertial sensors (Xsens, The Netherlands) attached above poll, withers, sacrum and tubera coxae. Variables were compared across movement directions and gaits using repeated measures ANOVA (parametric datasets) or Friedman Test (non-parametric datasets).

Results: Changes in foot placement relative to body position and orientation were small for both gaits in cranio-caudal and medio-lateral direction (maximum effect size at walk/trot: 66/102 mm). The amount of systematic movement asymmetry on the circle was comparable between walk (mean VS head/sacrum: 36/15 mm) and trot (30/15 mm, respectively), despite a 3-fold higher centripetal force during trot.

Conclusions: Foot placement relative to body position and line of travel does not differ markedly between straight line and circle and cannot solely account for the observed upper body movement asymmetry.

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Since movement asymmetry was comparable at walk and trot (despite differences in adopted movement pattern), centripetal force may not be the primary variable responsible for movement asymmetry on the circle.

Ethical Animal Research: The study was approved by the MSU Institutional Animal Care and Use Committee, protocol #06/11-112-00. **Sources of funding:** Sandra Starke's collaborative research visit for this project was funded by the British Society for Animal Science (BSAS), Murray Black Award. **Competing interests:** none.

BREEZING THOROUGHBRED HOOF ACCELERATIONS ON DIRT AND SYNTHETIC SURFACES

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Introduction: Racetrack surface type has been shown to affect horse injury rates, limb kinematics, and ground reaction forces. Understanding hoof-surface interactions may direct surface design to reduce the incidence of musculoskeletal injury. Hoof accelerations have been recorded at the trot on different surfaces. This study extends these analyses to racing gallop with quantified surface properties.

Methods: Left fore-hoof accelerations (triaxial, ± 500 g, 1000 Hz) were collected during gallop (10–16 m/s, 5 Thoroughbred racehorses) on dirt and synthetic surfaces of known shear strengths (shear vane). Accelerometric vectors were filtered (5th order Butterworth, stance 85 Hz, swing 30 Hz). Peak accelerations and stance phase durations (limb preparation, hoof landing {heel-strike and slide}, support, and grab) were compared using a mixed model analysis of covariance with surface, lead, stride frequency, and interaction fixed effects, and horse random effect.

Results: Peak dorsopalmar accelerations were 40% greater during landing on the synthetic surface ($P = 0.06$). Grab phase was 32% shorter on the synthetic surface ($P = 0.02$), with 34% less dorsopalmar impulse/mass ($P<0.01$). Previously reported failure shear stress of the synthetic surface was 20% greater, compared to dirt (normal stress >400 kN/m2, equivalent to trotting racehorse).

Conclusions: Dorsopalmar hoof accelerations were greater on the surface with greater shear strength (synthetic). The finding that accelerations were greater on the synthetic surface compared to dirt is in contrast to that of trotting horses on all-weather waxed and crushed sand surfaces. This discrepancy may be due to differences in horse gait, but also large variability in surfaces. Consequently, it is important to quantify surface behaviors to understand the effect of surface design and maintenance on limb biomechanics and propensity for injury.

Ethical Animal Research: Study protocols were approved by the University of California, Davis Institutional Animal Care and Use Committee and owner informed consent was obtained for all study horses. **Sources of funding:** Grayson Jockey Club Research Foundation Inc., Southern California Equine Foundation. **Competing interests:** none.

DISTAL FORELIMB KINEMATICS DURING THE EXTENDED TROT OF DRESSAGE HORSES RIDDEN ON TWO DIFFERENT ARENA SURFACES

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Introduction: Suspensory apparatus injuries are common in dressage horses. Studies demonstrate that surface type affects hoof forces and distal limb kinematics of equine athletes. Because synthetic race surfaces have lower stiffness and limb loads than dirt surfaces, it was hypothesized that dressage horses have less fetlock hyperextension on a synthetic surface than a dirt surface.

Methods: Two-dimensional, high-speed videos (250 Hz, 2 ppcm) of 6 dressage horses (ages 8–13; 5 males, 1 female) during extended trot on dirt and synthetic surfaces (6 trials (strides) per horse per surface) were digitized (low-pass Butterworth filter, 15 Hz). Fetlock and solar hoof angles determined from skin and hoof markers were compared between surfaces using a mixed model ANCOVA that accounted for horse velocity (saddle billet marker) and repeated horse measures. Surface mechanical behaviors were characterized using a track testing device. Maximum force, time to maximum force, maximum displacement, resting rebound, maximum vertical rebound, average stiffness, and maximum load rate were compared between surfaces and time of day (morning, afternoon) using a general linear model ANOVA.

Results: Maximum and toe off fetlock angles were greater on the synthetic surface compared to dirt ($\Delta = 2°$, $P = 0.070$). Mean maximum fetlock angle on the synthetic surface was 260°. The maximum load rate of the synthetic surface was 5 times greater than the dirt surface ($P = 0.0086$).

Conclusions: Synthetic surfaces were designed to reduce injuries in equine athletes. Because suspensory apparatus elongation is largely dependent on fetlock extension, it is possible that suspensory apparatus strain is greater on synthetic surfaces. Thus, surface design may require more refinement to promote injury prevention based on horse discipline and gait.

Ethical Animal Research: The University of California, Davis Institutional Animal Care and Use Committee reviewed and approved the protocols used in the study. Owner informed consent was obtained for all study horses. **Sources of funding:** Merial (A Sanofi Company) in association with the Student Training in Advanced Research (STAR) program, School of Veterinary Medicine, University of California, Davis and the Endowed Research Funds of School of Veterinary Medicine, University of California, Davis. **Competing interests:** none.

EFFECT OF LUNGEING AND CIRCLE SIZE ON MOVEMENT SYMMETRY IN SOUND RIDING HORSES

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Introduction: Evaluation of the movement during trot in circles is commonly used in clinical equine lameness examinations to exacerbate mild or bilateral lameness. Sound horses trotting in a straight line have a high degree of movement symmetry, which decreases with lameness. The aim of this study was to investigate the effect of lungeing and circle size on movement symmetry in sound horses.

Methods: Twenty five riding horses categorized sound by inspection on straight line and lungeing were trotted in a straight line and in circles of 8, 10, 12, 14 and 16 metres diameter on both reins. Overall symmetry (S), symmetry of diagonal limb pair loading (A), and symmetry of diagonal limb pair timing (W) were calculated from trunk accelerations measured over the 13th thoracic vertebrae (T13) with a 3-axis accelerometer. Data were analysed via ANOVA with horse as random effect.

Results: S, A, and W showed high degree of symmetry of straight line trot with mean ± SE = –6.0 ± 0.1, 0.01 ± 0.02 and –0.001 ± 0.003, respectively, that decreased with circular trot ($P<0.02$ in pairwise tests). S and A showed decreasing symmetry with decreasing circle size. For 16 and 8 metres, respectively; S = –5.2 ± 0.1 and –4.7 ± 0.1; A = 0.10 ± 0.01 and 0.16 ± 0.01; W = –0.009 ± 0.002 and –0.012 ± 0.002.

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Conclusions: In sound horses, movement symmetry measured over T13 decreased with circular trot and decreasing circle size as shown in earlier studies of movement of the poll and sacrum. The asymmetry mimicked inside diagonal lameness. Effect of circular movement and circle size should be considered in interpretation of lameness signs and asymmetry measured during circular trot.

Ethical Animal Research: The study was approved by the department ethical committee. The horses belonged to the Danish army, who gave their informed consent. As no invasive procedures were applied approval from the Danish Animal Experimental board was not needed. **Sources of funding:** Foreningen KUSTOS af 1861. **Competing interests:** none.

THE EFFECT OF PERINEURAL ANESTHESIA AND HANDLER POSITION ON LIMB LOADING AND HOOF BALANCE OF THE VERTICAL GROUND REACTION FORCE IN SOUND HORSES

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Introduction: To facilitate interpretation of limb loading and hoof balance obtained by pressure plate analysis in lame horses, the effects of handler position and perineural anesthesia in sound horses need investigation.

Methods: Six sound Warmblood horses were walked and trotted over a pressure plate, with the handler at the left and subsequently the right side, and before and after a bilateral palmar digital nerve block. One week later, this procedure was repeated before and after a bilateral abaxial sesamoid nerve block. Peak vertical force (PVF), vertical impulse (VI) and stance time (ST) of 5 hoof prints of both forelimbs were obtained (126 Hz). Asymmetry ratios were calculated.

Toe-heel and medio-lateral hoof balance curves of the vertical force were calculated. Mixed model ANOVA was used for statistical analysis of asymmetry ratios ($\alpha = 0.05$). Hoof balance curves were assessed qualitatively.

Results: There were no significant effects of handler position and perineural anesthesia on PVF and VI; small but significant effects of perineural anesthesia on ST at walk were associated with variability in horses' speed between sessions at walk ($P = 0.046$); no significant differences at trot. Hoof balance curves were not affected by handler position or perineural anesthesia, and were highly repeatable within each individual horse.

Conclusions: The lack of any effect of handler position or perineural anaesthesia on limb loading and toe-heel and medial-lateral hoof balance in sound horses makes it probable that significant aberrations found using pressure plate analysis are related to abnormalities and hence may be relevant in lameness diagnosis.

Ethical Animal Research: The study was approved by the ethical committee of the Faculty of Veterinary Medicine of Ghent University (n° 2012/151). **Sources of funding:** Ghent University. **Competing interests:** none.

EFFECT OF WEIGHTED BOOTS USED WITH DIFFERENT SHOEING STYLES ON LIMB MOVEMENT AND GROUND REACTION FORCES IN ICELANDIC HORSES AT WALK, TÖLT AND TROT

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Introduction: Weighted boots and unnaturally high and long hooves (S_H) are used to improve the 4-beat rhythm of the tölt and enhance forelimb action.

Methods: The effect of these methods on loading and movement of the limbs was investigated in thirteen Icelandic horses at walk, tölt and trot on an instrumented treadmill. Vertical ground reaction forces and kinematic data of fore- and hindlimbs were measured. Four conditions were differentiated: S_N (standard shoeing) and S_H, without and with weighted boots of 240 g (WB_{240}), respectively. Differences were tested using one-way repeated measures ANOVA, separately for each gait ($P<0.05$).

Results: At the tölt, in both shoeing styles, WB_{240} increased protraction height of the forelimbs by about 13%. The weight gain of 273 g at the distal limb induced by changing S_N to S_H had a similar and cumulative effect. Hoof length alone was also positively, but not significantly, associated with protraction height; the combination of S_H and WB_{240} resulted in an increase of up to 35%. Lateral coupling (pace-like rhythm) in tölt was reduced only with a combination of S_H and WB_{240}. Stride impulse increased with a change of hoof conformation alone at tölt, and combined with weight at all three gaits. Shorter relative stance durations in the forelimbs particularly at faster speeds resulted only in a minor increase of peak forces (+1.4%) due to an impulse shift to the hindlimbs.

Conclusions: Weight, particularly in combination with S_H, improved gait performance and marginally increased limb peak forces. However, enhanced inertial forces during swing might additionally stress internal distal limb structures.

Ethical Animal Research: The experiment was carried out with the approval of the Animal Health and Welfare Commission of the Canton of Zurich (No. 206/2010). Owner written informed consent was obtained. **Sources of funding:** Stiftung Forschung für das Pferd, the Haldimann Stiftung, the Swiss Metall Union, the Stiftung Temperatio, the Swiss Veterinary Office (FVO) and the Islandpferde-Vereinigung Schweiz. **Competing interests:** none.

IS SUBJECTIVE GRADING OF JUMPING TECHNIQUE RELATED TO MEASURABLE KINEMATIC PARAMETERS DURING FLIGHT?

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Introduction: There has been limited evaluation of biomechanical parameters during flight and their relationship to subjective assessments of jumping technique in ridden show-jumping horses.

Methods: Ten mixed breed horses (9.1 ± 1.9 yrs) were assessed at a British Equestrian Federation (BEF) World Class Performance three day training session. Riders received coaching during warm-up and after jumping a 15-fence (1.35–1.45 m) course once on day one and twice on day two; the second round on day 3 was an 8-fence jump-off. Two-dimensional high-speed motion-capture (250 Hz) was used to determine limb and back angles at 25%, 50% and 75% of the flight phase over four fences using markers placed at pre-determined locations. Technique

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grades (1–10) were assigned per fence by an international coach. Technique grades and kinematics were correlated using a Spearman's rank correlation test.

Results: Forelimb and hindlimb take-off distance and technique grade (4–7/10) were positively correlated (r = 0.29, 0.27, P≤0.01). Thoracolumbar angle (range 178–184°) at 25% and 75% of flight and neck/trunk angle (NT) at 50% and 75% of flight were negatively correlated with technique grade (r = -0.20 {P = 0.04}, -0.20 {P = 0.03}, -0.21 {P = 0.03}, and –0.28 {P<0.01} respectively).

Conclusions: Only weak correlations were evident, however, the findings suggest that increased take-off distance may be desirable for improvements in subjectively evaluated technique. The relationship between TL extension and improved technique grades may be explained by improved back stabilisation. Decreased NT and TL extension at 75% flight could suggest that back stabilisation and raising the neck in preparation for landing are associated with better jumping technique. Subjective grading of jumping technique appears to be influenced by kinematic features during flight.

Ethical Animal Research: The Royal College of Veterinary Surgeons confirmed that the procedures carried out were not Controlled Procedures under UK animal experimentation legislation. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** The World Class Development Programme is funded by the Department for Culture, Media and Sport, through Exchequer and National Lottery funds, via UK Sport. **Competing interests:** none.

JUMPING TECHNIQUE ALTERS LIMB KINEMATICS IN SHOWJUMPING HORSES

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Introduction: Showjumping (SJ) horses are at increased risk of distal forelimb soft tissue injuries; generally attributed to limb loading on landing. This study investigated relationships between the back, trailing limb angles at take-off, in flight and at landing.

Methods: Eight showjumpers jumped a 140 cm vertical obstacle (part of a 15-fence course). Two-dimensional high-speed motion-capture (250 Hz) was used to measure limb and regional back kinematics in the dorsoventral plane from markers placed at predetermined locations. Pearson's correlation tests were used to determine associations between limb and back angles at take-off, in flight and upon landing.

Results: All markers were clearly visible throughout. Greater head-neck ventroflexion at take-off was associated with decreased hock flexion (r = -0.81, P = 0.0141), and decreased neck-trunk angle on landing (r = 0.77, P = 0.03). Greater head-neck ventroflexion at landing, 50% and 75% flight was associated with decreased carpal extension on landing (r = 0.81, 0.84, 0.73; P = 0.02, 0.01, 0.04 respectively). Increased thoracolumbar and lumbosacral flexion at take-off was associated with decreased metatarsophalangeal joint extension upon landing (r = 0.78, 0.75; P = 0.03). Greater lumbosacral flexion at 50 and 75% flight (r = -0.73, –0.77; P = 0.04, 0.03 respectively) and greater thoracolumbar flexion at 25, 50 and 75% flight was associated with decreased elbow flexion on landing (r = -0.87, –0.78, –0.76; P = 0.01, 0.02, 0.03 respectively).

Conclusions: Decreasing head-neck angle and increasing thoracolumbar and lumbosacral flexion at take-off and in flight appeared to alter peak joint flexion and extension on landing. This suggests that optimising jumping technique in training may be beneficial to reduce the strain placed on the limbs at landing, which could potentially be valuable given the risk of distal limb soft tissue injuries in SJ horses.

Ethical Animal Research: The Royal College of Veterinary Surgeons confirmed that the procedures carried out were not Controlled Procedures under UK animal experimentation legislation. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** The World Class Development Programme is funded by the Department for Culture, Media and Sport, through Exchequer and National Lottery funds, via UK Sport. **Competing interests:** none.

EFFECTS OF SHOEING ON BREAKOVER FORCES IN ICELANDIC HORSES AT WALK, TÖLT AND TROT

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Introduction: To enhance expressiveness and regularity of the tölt, Icelandic horses are commonly shod with excessively high and long hooves. The aim of this study was to objectively assess the effect of shoeing on distal interphalangeal (DIP) joint moments of the left forelimb during break-over at walk, tölt and trot.

Methods: Twelve sound Icelandic horses accustomed to exercise with a rider on a treadmill were assessed with hooves trimmed and shod according to the standards of normal shoeing and with long and high front hooves; besides the 20 x 8 mm steel shoes, this condition included pads and packing material used in competition. Vertical ground reaction forces (GRFz) were measured with an instrumented treadmill. Time of break-over was determined kinematically. Measuring systems were started synchronously; sampling frequency was 480 Hz. Measurements were taken at 1.34 ± 0.04 m/s (walk), 3.89 ± 0.10 m/s (tölt), and 3.90 ± 0.10 m/s (trot). The DIP joint moment arm was determined on size-calibrated latero-medial radiographs of the left front hoof. Differences were tested using a t-test (walk) or repeated measures ANOVA (tölt, trot).

Results: In the condition with the long and high hooves, DIP joint moment was increased by 18.5% at walk (P<0.001), 17.3% at tölt (P<0.001) and 13.4% at trot (P = 0.017) although vertical GRFz at break-over was not statistically different between the two shoeing conditions. The increase could be attributed to the 22.7% increase of the lever arm.

Conclusions: High hooves with long toes may have negative implications for the health of the palmar structures of the distal foot.

Ethical Animal Research: The experiment was carried out with the approval of the Animal Health and Welfare Commission of the Canton of Zurich (No. 206/2010). Owners gave informed written consent. **Sources of funding:** Stiftung Forschung für das Pferd, the Haldimann Stiftung, the Swiss Metall Union, the Stiftung Temperatio, the Swiss Veterinary Office (FVO) and the Islandpferde-Vereinigung Schweiz. **Competing interests:** none.

INTERNAL FOOT KINEMATICS USING HIGH-SPEED FLUOROSCOPY

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Introduction: The navicular bone (NB) and the deep digital flexor tendon (DDFT) are common sites of lameness. Greater knowledge of foot mechanics will aid in understanding of the aetiopathogenesis and may provide new opportunities for treatment and prevention. It was hypothesised that changes in the distal interphalangeal joint (DIPj) and movement of the NB and DDFT during the stance phase will lead to changes in the moment arm of the DDFT around the DIPj.

Methods: Internal hoof kinematics were determined using a high-speed fluoroscopy system. Six horses were walked and trotted over a raised

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platform consisting of four force plates, through the field of view of the fluoroscopy system. The angles of the DIPj and the DDFT around the navicular bone and the moment arm of the DDFT were measured. Changes in these parameters during stance and differences between strides, gaits and horses were assessed using an ANOVA or paired T-test as appropriate ($P<0.05$).

Results: The range of motion (ROM, mean ± SD) during stance of the DIPJ was 28.6 ± 4.6° (walk) and 26.5 ± 6.3 (trot) with significant differences between gaits, strides and horses ($P<0.001$). The angle change of the DDFT around the NB was 21.9 ± 6.0° in walk and 26.6 ± 6.5 in trot. The change in moment arm of the DDFT around the DIPj was 3.7 ± 1.3 mm in walk and 4.1 ± 2.2 mm in trot. There were no significant differences between the different time points during stance or between gaits.

Conclusions: High-speed fluoroscopy is a novel method that allows assessment of internal foot kinematics. The observed changes between gaits during stance may result in altered stress distribution in the DDFT. Further studies will evaluate the effect of farriery intervention on internal foot kinematics.

Ethical Animal Research: this study was approved by the authors' institution's ethics and welfare committee. **Sources of funding:** Royal Veterinary College. **Competing interests:** none.

BIOMECHANICAL CONSEQUENCES OF UNEVEN FOREFEET AND HOOF CONFORMATION IN RIDING HORSES

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Introduction: The aims of this study were to quantify the morphological differences between uneven feet, and to evaluate the biomechanical locomotor consequences compared to horses with even feet, considered as normal.

Methods: Eight anatomical parameters that quantified conformational differences in the distal forelimbs of horses with a varied range of hoof asymmetries (n = 36, of which 2 were lame) were compared using discriminant analysis. Forefeet were subjectively scored for unevenness by one clinician. Kinetics and distal limb kinematics of clinically sound horses (n = 34; the 2 lame horses were excluded) were collected at trot and compared between even (n = 13) versus uneven (n = 21) forefeet and between all feet when classified as flat, medium and upright using MANOVA/ANOVA. The relative influence of contralateral differences in hoof angle and of absolute hoof angle on functional parameters was analyzed by multiple regression ($P<0.05$).

Results: Unevenness was best determined by the differences in dorsal hoof angle between the forefeet. In uneven footed horses, the flatter foot showed a significantly larger maximal horizontal braking and vertical ground reaction force, a larger vertical fetlock displacement and a suppler limb spring. A steeper hoof angle was linearly correlated with an earlier braking-propulsion transition. No significant differences were found between individual flat, medium or upright feet.

Conclusions: The conformational differences between the forefeet were more important for loading characteristics than the individual foot conformation. The contralateral differences in vertical force could in fact imply an early, subclinical sign of lameness developing in the steeper forefoot, as these differences were smaller than those reported for subtle, clinically evident lamenesses.

Ethical Animal Research: Horse owners gave informed consent. **Sources of funding:** FWO grant to S. Nauwelaerts, Research Grant Qualisys to S.J. Hobbs. **Competing interests:** none.

PHYSIOTHERAPY, REHABILITATION AND EQUITATION SCIENCE

SACROILIAC JOINT MORPHOLOGY: INFLUENCE OF AGE, BODYWEIGHT AND PREVIOUS BACK PAIN

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Introduction: Degenerative changes in sacroiliac joints (SIJ) are reported to cause poor performance in horses. The aim of this study was to document the frequency and nature of changes in SIJ surfaces of Thoroughbred (TB) racehorses and to correlate gross morphology and marginal new bone formation to age, body weight and history of back pain.

Methods: SIJ surfaces from 37 deceased TBs retired from the Hong Kong Jockey Club were photographed and measurements taken to facilitate subjective and objective analysis of gross changes. SIJ surfaces were described, and the surface area of the joint surfaces determined from the photographs, using Image J. Area and shape of the joint surfaces were recorded and SIJs were graded and allocated a type according to pre-determined criteria. Changes were compared for horses with and without clinical records of back pain.

Results: Mean age was 6.6 ± 2.3 years; weight 494 ± 35 kg; with no differences between horses with (n = 14) and without (n = 23) a history of back pain. There was no relationship between bodyweight ($r = 0.08$) or age ($r = 0.13$) and SIJ surface area. SIJs of the back pain group were affected by obvious gross pathology that showed certain characteristics. There was a relationship between surface area of joint and degree of marginal bone formation ($P<0.001$). SIJs from the group of TBs with no back pain had fewer and less obvious gross findings.

Conclusions: Clinical evidence of back pain, which may reasonably be expected to have affected the horse's racing performance, was associated with characteristic gross pathology of the SIJ.

Ethical Animal Research: Ethical approval for animal use was obtained by the institutional animal ethics committee (University of Queensland) and Hong Kong ethics committee. The study was performed on material collected during post-mortem examination. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Rural Industries Research and Development Council, Australia. **Competing interests:** none.

MEASUREMENT OF MOTION DURING MANIPULATION OF THE EQUINE PELVIS

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Introduction: Manipulation of the equine ilium and sacrum is utilised to assess range of motion at the SIJ. Motion of the ilium and sacrum during manual force application to the equine pelvis has been measured previously in vitro. The aim of this study was to measure the amount and direction of motion in vivo including comparison of bone fixated and skin mounted sensors.

Methods: Orientation sensors were skin-mounted over tuber sacrale (TS) and 3rd sacral spinous process (SP) of six standing Thoroughbred horses.

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Orientations of each TS and sacrum were recorded by one investigator during six trials of manual force applied to the pelvis, inducing cranial, caudal and oblique rotations. The study was repeated with the sensors mounted onto pins implanted into TS and SP. Mean values were reported in Euler angles for the three orthogonal planes lateral bend (LB), flexion-extension (F-E) and axial rotation (AR). Correlation between skin and bone fixated markers (Spearman R) was determined with significance set at P<0.05.

Results: The largest mean values recorded during rotations applied to the pelvises were for F-E (2.08 ± 0.35^{o}) with bone fixated sensors. Axial rotation gave the largest values recorded with skin-mountings (1.70 ± 0.48^{o}). There was poor correlation between skin-mounted and bone implanted markers.

Conclusions: Bony kinematics during external movement applied to the pelvis cannot be predicted from skin-mounted sensors, due to differences between skin- and bone-mounted sensors. Manipulation of the equine pelvis can be expected to produce motion in the plane of F-E, but axial movement should be interpreted with caution due to the potential effects of skin motion.

Ethical Animal Research: Ethical approval for animal use was obtained by the institutional animal ethics committee (University of Queensland). **Sources of funding:** Rural Industries Research and Development Council, Australia. **Competing interests:** none.

BACK-SHAPE CHANGES IN SPORTS HORSES

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Introduction: We have observed major back-shape changes over time in some horses, the speed of which may be influenced by work-type, skeletal maturity, nutrition and saddle-fit. Currently, there are no longitudinal data quantifying changes in back-shape and no objective data assessing the importance of each variable. The objectives were to: quantify back-shape changes over time; describe the effects of age, breed, weight, work-discipline, saddle-management and pain elicited on palpation.

Methods: Preliminary data from 98 sport horses in regular work in an on-going one-year longitudinal study. Weight and management changes were recorded. The thoracolumbar shape was measured at T18, T13 and T8 using a Flexible Curve Ruler [1] every two months. Ratios were calculated for each site for the widths 3 cm and 15 cm ventral to the dorsal midline. Differences in back-shape were determined using Wilcoxon signed-rank test. Chi-square test was used to assess associations between categorical variables, Spearman rank correlation for continuous variables, Mann-Whitney-U for continuous variables with binary outcomes and Kruskal-Wallis to assess ≥3 groups.

Results: T8 and T18-ratios were significantly different after two months (P<0.001) and T13 after four months (P = 0.004). Decreased ratio at T8 was related to ill-fitting saddles (P = 0.005), saddles tipping back (P = 0.020), pain on palpation (P = 0.032) and greater age (P = 0.004). Increased T18-ratio was associated with weight gain (P<0.001), more prevalent in stallions and geldings than mares (P = 0.035) and in dressage horses than other disciplines (P = 0.043).

Conclusions: Back-shape can change within two months, the speed and direction of which are influenced by saddle-fit, age and bodyweight. Pain elicited on palpation of the back may indicate that changes have occurred. Thus saddle-fit should be reassessed.

Ethical Animal Research: The study was approved by the Ethical Review Committee of the Animal Health Trust; there was informed owner consent. **Sources of funding:** Line Greve is the recipient of a Royal Veterinary College PhD stipend; we acknowledge financial support from World Horse Welfare. **Competing interests:** none.

Reference

[1] Greve L, Dyson S. An investigation of the relationship between hindlimb lameness and saddle slip. *Equine vet.J.* 2013, **45**,570-577.

MUSCLE FIBRE TYPE DISTRIBUTION OF THE THORACOLUMBAR AND HINDLIMB REGIONS OF HORSES: RELATING FIBRE TYPE AND FUNCTIONAL ROLE

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Introduction: The muscle fibre type (MFT) of the deep epaxial musculature has not been determined in horses. The objective was to determine the MFT distribution in selected muscles of thoracolumbar and hindlimb region of horses. This included deep epaxial and hypaxial muscles that were hypothesised to have a postural stabilising role. A second objective was to examine differences in MFT distribution between Arabians and Quarter horses.

Methods: Muscle biopsy samples (114) were obtained from 5 Quarter horses, 4 Arabians, and 2 Thoroughbreds. The myosin heavy chain distribution was determined by gel electrophoresis. Mann-Whitney rank test was used to compare the proportional MFT content and differences between breeds.

Results: Mm. sacrocaudalis dorsalis medialis and diaphragm had the highest proportion of MFT-I, 84.1% (±18.4) and 72.4% (±12.5), respectively. The remaining deep epaxial muscles and the hypaxial m. psoas minor had approximately equal MFT-I and II proportions. Mm. psoas major, iliocostalis, longissimus dorsi and the hind limb muscles contained mostly MFT-IIX: from 50.5% (±12.8) to 67.9% (±7.3). Quarter horses had significantly more MFT-IIX in m. psoas major 60.8% (±9.1) than Arabians 41.9% (±3.2). Arabians had significantly more MFT-I fibres in m. longissimus dorsi 16.9% (±4.6) than Quarter horses 9.9% (±1.4) (P<0.05).

Conclusions: The MFT distribution of the deep epaxial muscles, mm. psoas minor and diaphragm, suggest a postural stabilizing role in contrast to the fibre type proportions of the hindlimb muscles. The findings can direct diagnosis, prevention and treatment of muscular or neuromotor dysfunction in the thoracolumbar and hind limb area.

Ethical Animal Research: The study was approved by the University of Queensland Animal Ethics Committee. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** none. **Competing interests:** none.

THORACOLUMBAR FLEXION-EXTENSION DURING WATER TREADMILL WALKING

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Introduction: Water treadmill exercise is used for rehabilitating horses with back dysfunction, yet little is known about the effect of this type of exercise on back movement.

Methods: 8 horses walked in water level with the hoof, fetlock, hock and stifle. Reflective markers over the dorsal spinous processes of T6, T10, T13, T18, L3, L5 and S3 and left and right tuber coxae were used to measure flexion-extension range of movement (F-E ROM) around T10, T13, T18, L3 and L5, vertical displacement of the pelvis (Pelvic ROM) and vertical distance between T13 and L5 (lowest and highest point of the back). 3D coordinates were tracked using ProReflex cameras. 5–9 strides were analysed for each horse at each water height. Repeated measures ANOVA and post hoc tests were used to determine differences in measured variables between water heights (P < 0.05).

Results: Peak flexion of T10, T13, T18 and L3 occurred in hock height water. Peak extension of T18, L3 and L5 occurred in stifle height water without a significant difference in F-E ROM between water heights (P>0.05).

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Pelvic ROM was greatest in stifle height water, with no significant difference between water heights. The vertical distance between T13 and L5 was greatest in stifle height water (P<0.05 between hoof and hock height water) as a result of an elevation of L5 relative to T13.

Conclusions: As water height increases L5 is elevated relative to T13, increasing the tendency for extension of the thoracolumbar spine. To maximise thoracolumbar flexion and avoid extension, water at hock height is more beneficial then stifle height water.

Ethical Animal Research: Project approved by Hartpury College Research Ethics Committee. **Sources of funding:** Hartpury College. **Competing interests:** none.

CHANGES IN MOVEMENT SYMMETRY DURING LONG-TERM EXERCISE IN HORSES

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Introduction: Asymmetry of the trot may occur or increase during endurance rides due to lameness, pain or fatigue. By visual inspection of the movement it is difficult for veterinarians, riders and researchers to distinguish between fatigue and low-grade lameness, which make objective movement analysis relevant for this purpose. The aim of this study was to determine changes in movement symmetry during long-term exercise.

Methods: Movement symmetry of 45 horses finishing endurance rides of 120–160 kilometres was determined by tri-axial accelerometry according to an earlier published method. Data was collected in Denmark and Germany from 5 international competitions between 2011 and 2013. Movement was assessed at trot on a straight-line in-hand on the day before the race and 30 mins to 3 hrs after passing the final veterinary examination. An overall symmetry index S was calculated on the basis of trunk accelerations measured at 240 Hz during 8 strides. All trotting sequences were recorded on videotape. A paired t-test was performed comparing symmetry indices before and after racing for each individual horse.

Results: A significant decrease in post-ride symmetry was detected (P = 0.0042). This is seen as a lower S-index before racing (-6.18; SD = 0.86. CI [-6.427; –5.923]) compared to after racing (-5.67; SD = 0.79; CI [-5.908; –5.434]).

Conclusions: This study indicates that the biomechanical method applied allows quantification of the symmetry of movement during field conditions. Most horses had decreased movement symmetry after the race. Accordingly the study provides the basis for further investigation of the causes, including surface, climate and fitness; and the possible health consequences of asymmetric movement developed during long-term exercise.

Ethical Animal Research: The study was pre-approved by the FEI, the local organizing committees, ground jury, and Danish riding association (DRF). Participation was voluntary and the owner or rider signed an informed consent. All information regarding the horses is kept confidential and the horses coded for anonymity. **Sources of funding:** This study is part of a university and government funded PhD project. Private funding from “Hesteafgiftsfonden” and KDH (Royal Danish Horse Insurance). **Competing interests:** none.

CROSS-SECTIONAL AREA MEASUREMENT OF CERVICAL SPINAL MUSCLES

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Introduction: In people with neck pain, ultrasonography (US) can measure atrophy and response to physiotherapy in peri-vertebral muscles of the cervical spine, i.e. m. multifidus and m. longus colli. This study compared the reproducibility of muscle cross-sectional area (CSA) measurements made by US and MRI (the gold standard) at different levels in the equine cervical spine.

Methods: From US and MRI images of three mature pony cadavers, CSA was calculated at vertebral levels C3-C6 (m. multifidus) and C2-C5 (m. longus colli). ANOVA analysis compared US and MRI image acquisition reliability (n = 360) and CSA (n = 1080) among subjects and vertebral levels.

Results: For image acquisition of m. multifidus (C3-C5), the ICC (95% CI) for MRI and US was 99% and 61%, respectively. Image acquisition was unreliable at C6. CSA calculation did not differ between MRI and US. For m. longus colli (C3-C5), the ICC for image acquisition was 100% and 88% for MRI and US, respectively. Image acquisition was unreliable at C2. The ICC for CSA calculation was 98% for MRI and 100% for US. For both muscles, the mean CSA was largest at C4 and C5.

Conclusions: Based on these results, ultrasonography could be used in the live horse for reliable CSA measurement of m. multifidus and m. longus colli in the mid-cervical spine. This technique may advance knowledge of causes and effects of neck pain in horses.

Ethical Animal Research: Approval for this study was obtained under Institutional Animal Care and Use Committee number 02-11/020-00. The study was performed on material collected during post-mortem examination. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Michigan State University CVM Endowed Research Funds. **Competing interests:** none.

INTRA-OPERATOR AND INTER-OPERATOR RELIABILITY AND REPEATABILITY OF MUSCLE MEASUREMENT VIA ULTRASONOGRAPHY IN THE EQUINE CERVICAL SPINE

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Introduction: Ultrasonography is used for functional assessment of deep peri-vertebral muscles in people with neck pain. This study evaluated intra-operator and inter-operator reliability and repeatability of ultrasound image acquisition (US) and muscle cross-sectional area measurement (CSA) of m. multifidus and m. longus colli in the equine cervical spine.

Methods: Two US operators independently acquired 60 unilateral US images per horse from 5 standing horses (mean ± SD: age: 12.1 ± 10.5 years) at spinal levels C3-C6 (m. multifidus) and C2-C5 (m. longus colli). Each operator traced each image blindly three times (n = 360 per operator) to calculate CSA. ANOVA analysis compared CSA across all subjects within and between operators for effects of vertebral level, reliability of image acquisition and repeatability of CSA calculation.

Results: Intra-operator results for m. multifidus were 72% (ICC) and 5% (CV), and 90% (ICC) and 4% (CV) between operators. Image acquisition was unreliable at C6. Intra-operator results for m. longus colli were 100% (ICC) and 0% (CV), and 77% (ICC) and 0% (CV) between operators. Image acquisition was unreliable at C2. CSA for both muscles was largest at C4 and C5.

Conclusions: Ultrasonography could be used reliably and repeatably for CSA measurement of m. multifidus and m. longus colli in the mid-cervical

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spine of the horse. Further studies will determine reliability across time and CSA side-side symmetry in horses with neck pain.

Ethical Animal Research: Approval for this study was obtained under Institutional Animal Care and Use Committee number 02-11/020-00. **Sources of funding:** Michigan State University CVM Endowed Research Funds. **Competing interests:** none.

IS THERE A RELATIONSHIP BETWEEN TAIL CARRIAGE AND LAMENESS IN HORSES?

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Introduction: Tail deviation in horses is anecdotally viewed as an indicator of lameness. Compensatory mechanisms in lame horses are well documented, however there is no information published on the relationship between lameness and tail carriage in horses. The objectives of this study were to quantify differences in tail carriage in relation to the type of lameness (front/hind, left/right) and to establish a relationship with confounding variables.

Methods: Videos of 150 horses presented for lameness examination were evaluated. Tail carriage angles were measured in 50 horses and subjectively assessed using a categorical 4-point scale in 100 horses. Lame leg(s), lameness score(s), age, sex, breed, duration and cause of lameness were recorded. Differences in tail carriage between lameness types were assessed using paired T-tests (continuous data) or chi-square tests (categorical data). Logistic regression evaluated the effect of degree and duration of lameness, and age on degree of tail deviation.

Results: 87.2% of the horses showed some degree of tail deviation. The mean ± SD tail angle was 92 ± 14.5° (90° = midsagittal) for forelimb and 87 ± 11.2° for hindlimb lameness. The mean ± SD tail angle was 91.1 ± 17.3° for right-sided and 89.5 ± 16.9° for left forelimb lameness; 89.8° ± 8.8 for right and 86.5°± 9.8 for left hindlimb lameness with no statistically significant difference between front/hind ($P = 0.52$) or left/right lameness ($P = 0.237$). No statistically significant correlation was found between degree and duration of lameness, age of horse and presence or degree of tail deviation.

Conclusions: No significant relationship between tail carriage and lameness was identified in this study and tail carriage should therefore not be considered an indicator for lameness.

Ethical Animal Research: This study was approved by the authors' institution's ethics and welfare committee. Explicit owner informed consent for participation in this study is not stated. **Sources of funding:** Royal Veterinary College. **Competing interests:** none.

VERTICAL DISPLACEMENT OF THE EQUINE PELVIS WHEN TROTTING ON AN AQUA TREADMILL

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Introduction: Hydrotherapy is increasingly applied within the equine industry for rehabilitation and therapy. Substantial anecdotal evidence exists suggesting the benefits of its application but while there is extensive literature on the use of aqua-treadmills in humans and canines there is comparatively little in equines. Therefore, limited research exists supporting the role of the aqua-treadmill in current therapy practices. This study aimed to investigate the effect of water depth on pelvic movement during aqua-treadmill exercise.

Methods: Eight horses were trotted on an aqua-treadmill at four water depths specifically measured to individual horses (P3, fetlock, MC3, carpus). Pelvic displacement was recorded using an optical motion capture system to collect coordinates from markers placed on the tuber sacrale, tuber coxae, withers and lumbar spine. Data were processed using custom written scripts (Matlab®) and ANOVA was performed.

Results: All subjects demonstrated a significant increase in vertical displacement of the pelvis as water depth increased from P3 to carpus while displacement symmetry was not significantly altered with a change in water depth.

Conclusions: The common practice of increasing water depth to intensify the physical demands of the exercise while maintaining the desired movement symmetry is supported. Having knowledge of how a horse moves on an aqua-treadmill is vital for tailoring specific therapy in order to most successfully rehabilitate the horse from injury. Investigation of the effects of increasing water depth on asymmetric horses should be carried out to further support its application as a tool for rehabilitation.

Ethical Animal Research: Ethical approval was obtained from the Ethics Review Committee at Moulton College. Owner consent was granted for all subjects and all horses were handled in compliance with British Horse Society standards throughout the trials. **Sources of funding:** Thomas Harrison Trust at Moulton College. **Competing interests:** none.

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