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Foreword and Acknowledgements

The mission of the International Conference on Equine Exercise Physiology (ICEEP) is to advance the understanding of the physiology, function and health of athletic horses through the promotion of scientific research. The abstracts in this compilation represent a collation of novel research presented at the tenth quadrennial meeting, which returned to Australia after 24 years. Befitting current industry around the world, the abstracts cover a wide range of topics relevant to athletic horses with a particular emphasis on the major systems critical to excellent performance in the horse such as biomechanics, training, and cardiorespiratory phenomena. Reflecting progressive evolution in medicine and technology, the compiled abstracts also instruct on new findings in genomics, physiotherapy, drugs, nutrition and other salient issues.

The goal of the International Committee of ICEEP in publishing these abstracts is to disseminate current scientific information to veterinarians, physiotherapists, trainers, owners, and riders. It is our sincerest wish that these actions will foster attention to and advances in equine health, welfare and performance. We hope to achieve this by promoting collaborative interdisciplinary research efforts, the mentoring of students and junior scientists, and the sharing of validated information to all who might benefit and in turn be better able to support these supreme athletes.

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Racing Victoria

Applied Physiology – Training

Recovery heart rates as a predictor of race position in National Hunt Racehorses

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Prediction of race fitness using the principles of excess post-exercise oxygen consumption is a potentially valuable applied exercise physiology tool. We hypothesised that horses with a lower recovery heart rate after a standardised field test would perform better in their subsequent race. Twenty mature, experienced National Hunt horses (15 geldings, 5 mares; 6.5±1.1 years; 489±33.5 kg), underwent 34 pre-race standardised 3-interval field exercise tests using telemetric heart rate (HR) and global positioning satellite (GPS) monitoring on a 1,400 m track inclined 32 m. Horses were classified into three groups based on 1 min post-exercise HR after interval 3 (>140 bpm; unfit [U]; 120-140 bpm; fit-to-race [FR]; <120 bpm; fully fit [F]). All horses were from the same yard, under the same management and in their final stage of training (race-ready). Horses were excluded if they were lame or clinically unwell. The outcome measure of finishing in the top third of the field was compared to classification using 2×2 tables (Statcalc, EpiInfo). Peak HR, speed and 1 min post-exercise HR were 213±5 bpm, 49.3±1.8 kph and 125±16 for interval 3 respectively. Horses classified as U (n=8) did not race. F and FR horses competed in 26 jump races (23 hurdles, 3 bumper; 3,200-5,000 m). Horses classified as F (n=16) were more likely to finish in the top third of the field than FR (n=10) (OR 12.0; 95%CI 1.8-81.7; *P*=0.01). We conclude that post exercise heart rate following standardised interval exercise is a predictor of race position in NH racehorses and a useful guide for trainers.

The effect of treadmill training on equine muscle morphometrics and muscle metabolomics

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The aim of this study was to evaluate the effect of 8 weeks treadmill training (TT) on skeletal muscle diameter and muscle metabolic profile. Seven untrained Friesian horses completed an 8-week TT program (20 min/session, 5 days/week, speed 1.25 m/s, no incline). Morphometric assessment of 15 muscles was performed by ultrasound at the start and finish of the study. Muscle biopsies were harvested before and after 8 weeks of TT from the pectoralis profundus (PP) and vastus lateralis of the quadriceps femoris (QF). Metabolic profiling was performed by (RP)/UPLC-MS/MS and HILIC/UPLC-MS/MS and analysed by two-way ANOVA (P<0.05). Eight weeks of TT significantly increased the muscle diameter of the cervical (+57%) and thoracic (+26%) part of the trapezius, the PP (+29%), and brachiocephalicus (+10%), and significantly decreased the diameter of the QF (-19%), the lumbar part of the erector spinae (-9%) and the semitendinosus (-7%). TT significantly increased long chain acylcarnitines in both PP and QF muscles, while long chain fatty acids significantly decreased. Early (glucose, glucose-6-P and fructose-6-P) and late stage (pyruvate and lactate) glycolytic intermediates and pentose-P-pathway intermediates were significantly increased in the QF. A significant increase in oxidized glutathione, taurine and intermediates of the glutamine/glutamate metabolism and a decrease in glycine and acetyl-glycine was found in the PP. Metabolite profiling revealed an upregulation of fat oxidation and glycogen storage capacity in QF muscle (decreasing muscle mass) with upregulation of fat oxidation capacity and amino acid metabolism in PP muscle (increasing muscle mass).

Equine endurance race pacing strategy and performance in 160 km single-day races

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Race pace strategy has been studied extensively in human endurance sports, however the impact of pacing strategies in equestrian sport has not been widely investigated. This study analysed electronically-timed FEI 160 km (single-day) CEI*** endurance races across Europe, Africa and the Middle East, from 2011-2017. Retrospective competition records for 805 horses in 36 races, each consisting of 5 phases (loops), were evaluated; 52% (n=416) of horses completed the races analysed, with the remaining 48% (n=387) not finishing. Horses failed to complete due to gait abnormalities (n=231; 60%; 68% loops, 2, 3&4), metabolic problems (15%; n=57; 65% loops 3&4) or being retired on the course (13%; n=51; 75% loops 2,3&4). Average speed between finishers and non-finishers didn't differ (P>0.05) across the duration of the race, however, pacing strategies varied significantly from loop 2 to the finish (P<0.01) with finishers adopting a more consistent approach, 4.1% variance compared to 8%. Further analysis of completing horses (n=403) investigated if speed and pacing strategy differed between the top 3 horses and those in lower placings. Loop speed was increased for horses placed in the top 3 for all loops (P<0.02) except loop 5 (P>0.05), these horses also recorded 6.6% higher average speeds across the race (P<0.0001) compared to the other finishers. Horses in the top three also adopted more consistent pacing strategies, 3.2% variance compared to 5.8%. Multiple factors influence performance, but these results suggest consistent pacing strategies may be a successful mechanism to increase the chance of completion and success in endurance race.

Relative contribution of the aerobic and anaerobic energy systems to supramaximal equine exercise

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The objective was to determine relative aerobic and anaerobic (lactic and alactic) energy contributions to supramaximal exercise using two different methods. Prospective, randomised, controlled study; n=8. Fit horses performed a VO_{2max} test and three supramaximal treadmill runs (105, 115 and 125%VO_{2max}). Blood lactate (BL) was measured at rest, every 15 s during runs, and 2, 5, 10, 20, 30, 40, 50 and 60 min post-exercise. Method 1: Oxygen demand was calculated for each supramaximal intensity based on the VO_{2max} test, and relative aerobic and anaerobic contributions were calculated from measured VO_2 and the accumulated oxygen deficit. Method 2: Aerobic contribution was calculated using the trapezoidal method to determine VO_2 during exercise. A mono or bi-exponential model was fitted to the post-exercise VO_2 curve. Alactic contribution was calculated using the coefficients of this model. Lactate anaerobic contribution was calculated by multiplying the $\Delta BL_{Peak\text{-}Resting}$ by 3: 1 mmol/l BL increase=3 ml O_2/kg. A 2-way RM ANOVA plus Holm-Sidak post-hoc compared variables with >1 measurement at each timepoint. Relative contributions from each method were compared using a t-test ($P \leq 0.05$). Relative aerobic (P=0.25) and anaerobic (P=0.22) contributions were not different between methods. Horses' mean contributions were 81.4, 77.6 and 72.5% (aerobic), and 18.5, 22.3 and 27.4% (anaerobic; 105, 115, 125%). Individual alactic capacity was constant, irrespective of exercise intensity (P=0.33), and accounted for 0.11% of energy use. Relative energy contributions can be calculated using measured VO_2 and BL in situations where the exercise intensity is unknown. Understanding relative metabolic demands could help develop tailored training programs.

Training speeds and distances undertaken by Australian Thoroughbred Racehorses

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Musculoskeletal injuries (MSI) in racehorses are commonly due to bone fatigue; a function of the number of cycles (strides) and the magnitude of the load applied to the limb. In horses in training, load and number of cycles are a function of speed and distance, with galloping (>14 m/s) for greater than 6,000 m/month reported to increase the risk of fracture. As there is limited data on distances and speeds (workload) to which racehorses are exposed we aimed to determine training volumes undertaken by Australian Thoroughbred racehorses. Registered trainers (n=66) were interviewed about their pre-training, two-year-old and mature horse training practices. We assessed differences in median and variance in workloads between horse- and trainer-level factors. The volume of total gallop work varied substantially between trainers [median distance>13.3 m/s; 8,000 m/month (IQR 6,400-12,000 m/month)]. For gallops at speeds greater than 14.3 m/s, 50% of mature horse programs exceeded 6,400 m/month. Cluster analyses identified four workload programs: (1) low volume (4,800 m/month), (2) medium volume (8,000 m), (3) medium volume (12,800 m) with a higher proportion of high-speed workouts, and (4) high volume programs (19,200 m), with 23.4, 50.0, 17.2 and 9.4% of trainers predominately training racehorses under each of these programs respectively. There was substantial variation in workload volume between trainers and for different horse ages and targeted race distances (younger < older, sprinters < stayers). Australian Thoroughbred training programs include high volumes of galloping with more than half exceeding previously reported risk levels for MSI. Future work aims to determine injury risk and performance under different training programs.

A study of the gait and respiratory pattern of horses when swimming

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We aimed to describe the swimming gait of horses and to determine if respiration is coupled to swimming stride. Video and audio recordings were analysed for 18 race fit horses (14 Thoroughbreds and 4 Standardbreds) free swimming in a straight line without added resistance from current or tether, in three different pools. Limb timing, strides/min, time until first breath, breaths/min (RR) and mean stride to breath frequency when swimming were recorded and the correlation between strides/min and breaths/min determined using linear regression ($P<0.05$). Stride frequency (mean 48 strides/min, range 40-55), RR (mean 23.5 breaths/min, range 14-30) and strides/breath (mean 2.04, range 1.54-3.21) was variable between horses. No respiratory locomotor coupling was evident with no significant relationship between strides/min and breaths/min (r=0.089, $P=0.72$). Most (88%) of horses had a swimming gait described as a 'disconnected pacing action' or 'dog paddle' which was unlike the limb timing of any overground gait. The implications of this difference and the lack of any respiratory locomotor coupling when using swimming for training and rehabilitation of horses requires further study.

A survey of trainers on the use of swimming exercise for Standardbred racehorses in Australia

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The extent to which swimming is used for Standardbred racehorses, perceived benefits, and contraindications have not been reported and guidelines are not available. We aimed to investigate swimming practices used for Standardbred racehorses and determine targets for future research and educational initiatives. An electronic survey with 23 questions was sent to all trainers with email addresses in the Australian Harness Racing Directory and univariate analysis performed. Of 1,770 trainers contacted, 250 responded via the survey link (response rate 14.1%); 238 trainers with mean 31.1 years' experience (range 4-60 years) completed the survey adequately with 37.4% (89/250) using swimming exercise for mean 5.5±7.2 horses (range 1-35). The main reason for not swimming horses was lack of access to an adequate facility. Reasons for not swimming individual horses varied widely. Most trainers learnt to use swimming exercise from a mentor (72.7%) and/or by trial and error (32.9%). Free swimming was more common than tethered swimming and a dam was most commonly used. The most popular reasons for swimming were to replace track work for leg problems (76.1%), improve fitness (62.5%) and for 'mental freshness' or variety (55.7%). Swimming protocols varied widely with mean 4.2 (range 0.5-12) swim sessions per horse each week, of mean 9.3 min (range 1.5-30 min) per session mostly as a continuous swim, but sometimes as intervals. Trainer opinions and protocols vary widely with respect to swimming exercise in Standardbred racehorses. The role of swimming exercise requires further study so that evidence-based recommendations can be made.

Mitochondrial capacity is impaired in aged vs growing horses during submaximal exercise training

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Effects of age and exercise training on mitochondrial number (citrate synthase activity; CS), function (cytochrome *c* oxidase activity; CCO), and oxidative capacity (P) were evaluated by high-resolution respirometry in muscle from aged (n=9; 22±4.5 yr) and yearling (n=8; 9.7±0.7 mo) Quarter Horses. All horses were sedentary for at least 6 mo prior to this study, and the standardized exercise program was similar between groups. Triceps brachii (TB) and gluteus medius samples were collected at wk 0, 8, and 12 of exercise. Data were analysed using PROC MIXED in SAS 9.4 with age, time, muscle, and all interactions as fixed effects. Training increased CS (P=0.003) and integrated (per mg protein) CCO activities (P=0.011). Activity of CS was higher in aged than young TB (P=0.029), but intrinsic (per unit CS) CCO activity was lower (P=0.001), and integrated CCO activity tended to be lower (P=0.07) for aged horses across muscle groups. By wk 12, integrated P with complex I substrates (P_{CI}) increased in aged (P<0.003), but not young horse muscle. Integrated P_{CI} plus complex II substrates (P_{CI+II}) and complex II electron transport capacity (E_{CII}) increased by wk 8 for both groups (P<0.0005). Maximum E (E_{CI+II}) increased by wk 8 for aged (P=0.0001), but not until wk 12 for young horses (P=0.01). P_{CI}, P_{CI+II}, and E_{CI+II} were lower for aged than young horses (P<0.02). Exercise improved mitochondrial measures in all horses, but aged horses showed impaired mitochondrial function and oxidative capacity. These effects may have been compounded by growth in young horses.

Race-induced weight loss and its recovery time in Trotters: factors of variation

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Racing induces dehydration leading to variable weight loss and the time to recover weight after a race is important as it is a precondition to resume intensive training. The objective of this study was to analyse some factors related to race-induced weight loss and weight recovery in Trotters. In one stable of trotters, 28 competing horses aged 3 to 10 years were weighed daily over several years. Comparison of pre-race weight to 24 hours post-race weight allowed calculation of weight loss in the race (WL) as well as the number of days required to return to pre-race weight (recovery time: RT). These two variables were calculated from a total of 648 competitions. The influence of individual factors (horse, age and temperament) and environmental factors (transport time, running distance, type of race, and outside temperature) were evaluated by multiple regression analysis. The average (min-max) values of WL and RT were respectively -9.4 kg (0 to -26 kg) and 3.3 days (1 to 9 days). The age of the horse significantly decreased WL without influencing RT. A nervous temperament significantly increased WL and RT. High temperatures and long transport significantly increased WL but not RT, with interaction. Race types or race distance had no influence on WL and RT. Race induced weight loss and recovery time were influenced by individual factors such as age and temperament and also by environmental factors such as outdoor temperature or the duration of transportation.

Can an Equine Perceived Exertion scale monitor training loads in young Thoroughbred racehorses?

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Monitoring training loads of Thoroughbred racehorses is increasingly relevant as the industry becomes more competitive with an emphasis on improving welfare. The objective was to investigate the potential role of an Equine Perceived Exertion scale (EPE) as an estimate of training loads on Thoroughbred racehorses (TB). Over a period of twelve weeks in varying conditions, data were randomly collected from 30 two and three-year-old TB. Each were subjected to daily relatively low intensity training of walk, trot and canter whilst wearing heart rate monitors and global positioning system devices. Immediately following exercise, respiratory rate, behavioural characteristics, sweating, muscle engorgement, oral mucous membranes and capillary refill time were scored. The sum of scoring, derived from the authors' knowledge of physiological changes expected in horses following training, formed the total score and subsequent rating of EPE when a scale was applied. Data were analysed with descriptive statistics (mean±SD), one-way ANOVA and linear regression ($P<0.05$). There was a significant difference in duration of training between two and three-year-old TB with three-year olds training longer (20.0 to 70.9 minutes; $P=0.011$). Linear regression demonstrated a moderately strong positive relationship between Total Score and EPE ($r^2=0.911$, $P<0.05$) and between an individual's EPE rating and heart rate recovery ($r^2=0.740$, $P<0.001$). These findings may support the use of an equine perceived exertion scale as a means of monitoring training loads in TB racehorses.

Can human training load quantification be applied to equine training?

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Optimal training requires understanding of exercise variables to achieve enhanced performance and progression. Rating of perceived exertion scales (RPE) are validated as proxy measures for physiological workload in human sport alongside Edwards' training load. Edwards' methodology is an algorithm using duration of time within defined heart rate (HR) zones. Both methods are validated to monitor Training load (TL) in human athletes. This study investigated if these methods could potentially offer a simple and repeatable measure of workload in equine training regimens. HR data (Polar V800) were obtained during one single exercise session from 32 horses across a range of equestrian disciplines with experienced riders and trainers, on different surfaces. Based on Edwards' TL, duration (minutes) spent within five pre-defined training zones (1: <80; 2: 80-120; 3: 120-160; 4: 160-200; and 5: >200 bpm) were factored to obtain total TL (HRTL). Ratings (1:very,very easy-10:maximal) were collected from riders and trainers, reflecting horses' RPE for entire sessions, then multiplied by exercise duration to determine internal TL (INHR). Spearman's correlations ($P<0.05$) identified if relationships existed between HRTL and INHR. Statistically significant correlations between HR and INHR were found for both riders' and trainers' ratings of TL, riders: cohort: r=0.80, $P=0.0001$; sports-horses: r=0.81, $P=0.0001$; racehorses: r=0.72, $P=0.002$; and trainers: cohort: r=0.82, $P=0.0001$; sports-horses: r=0.89, $P=0.0001$; racehorses: r=0.94, $P=0.0001$. The results suggest HRTL and INHR can be used as inexpensive and easy tools to quantify TL and objectively assess progress in training. Exploration of optimal HR zone allocation for specific disciplines and breeds, alongside application of TL monitoring within equestrianism is warranted.

Failure to finish in a United Arab Emirates 160 km endurance ride

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Due to flatter terrain and a greater focus on competition (rather than completion), horse and rider teams participating in endurance rides hosted in the United Arab Emirates (UAE) typically compete at faster speeds than teams competing in North American rides. Using data obtained from the Dubai Equestrian Club website (www.dubaiequestrianclub), completion rates and causes of failure were compared over the years 2011-2016 for the annual 160 km HH Sheik Mohammed bin Rashid Al Maktoum Cup. A secondary objective was to investigate longevity of UAE horses by determining how many horses competed in this ride over multiple years. Descriptive statistics and Chi Square analysis were used to interrogate the data. Of 854 starting horse and rider teams, only 31% (range 21-42%) successfully completed the ride, with higher completion rates in 2015 and 2016 ($P<0.05$) than in 2011-2014. Elimination for lameness (32% overall, range 29-37%) was more common ($P<0.05$) than elimination for metabolic problems (13% overall, range 8-19%). There was no significant difference in elimination rates for lameness over the 6-year period but elimination rates for metabolic problems were greater ($P<0.05$) in 2011, 2013, and 2015. Of all horses starting in this UAE ride, 18% (annual range 13-23%) competed in the ride the following year while only 10% (annual range 8-12%) competed in the ride for 3 years in a row. These completion rates are lower than completion rates for North American 160-km rides despite similar causes for elimination. Additionally, the attrition rate of elite endurance horses competing in 160-km rides is high.

Acute and chronic workload; high chronic workload may decrease injury risk in elite eventing horses

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In human sport science the fitness-fatigue model is used to establish an athlete's preparedness for high performance. The aim of this project was to investigate whether acute and chronic workload calculations could be used to predict injury risk in elite eventing horses. Workload and injury data were collected from 58 international eventing horses (comprising CCIP** ponies (n=5); and 1*(n=18); 2*(n=14) and 3*(n=21)-level horses). In total 94 individual competition seasons were followed (n=35 participated 1 year; n=10 for 2 years and n=13 for 3 years). During this period, heart rate (HR; beat/min) and GPS data from all conditional training sessions and competitions were collected. A binary logistic regression was used to compare acute (1-week) and chronic (4-weekly) workloads between injured and non-injured horses for the GPS variables: total distance (TD), high-speed distance (HD=velocity between 6.6 and 9.5 m/s), and sprint distance (SD=velocity>9.5 m/s). In total 2,310 data sets were created; 1,908 training sessions and 402 competitions. An average training session consisted of TD=12,511 m, HD=3,227 m and SD=1,543 m. A total of 61 soft-tissue injuries were reported. Increased acute load TD in the previous week was associated with an increased likelihood of injury ($P<0.0001$). Soft-tissue injury risk was significantly increased when a high acute HD was combined with a high chronic TD ($P=0.002$), but an increase in chronic HD reduced the injury risk ($P=0.021$). In eventing horses, just as in human athletes, spikes of acute workload increased the risk of injury whilst a higher chronic workload may protect against injuries.

An overview of national level equine endurance competition in New Zealand

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Epidemiological studies have examined the sport of Endurance at the international (FEI) level. However, much participation occurs at a national level in preparation for FEI level sport. The aim of this study was to describe participation in endurance sport at the national level in New Zealand. Data were collated within a customised access database of all endurance competitions held in New Zealand between the 2010/11 and 2015/16 competition seasons. Data were tabulated and descriptive statistics derived using Stata v14. Data were collected on 7,493 starts; of which 6,887 starts (n=684 horses) involved horses registered with the national body. The horses had a median of 3 (IQR 1-6) starts per season. The median accumulated ride distance/season/horse decreased from 190 (IQR 60-380) in 2010/11 to 160 (IQR 80-303) km/horse in 2015/16. Ride entries were dominated by the 40 km (n=3,437, 46%) and 80 km distance (n=2,616, 35%). The percentage completions decreased as ride distance increased from 91% in 40 km rides to 51% in 160 km rides. The percentage of starters eliminated for metabolic reasons increased from 0.8% in 40 km rides to 9.2% in 160 km rides. Lameness accounted for more eliminations with 3.6% of starters in 40 km eliminated, increasing to 34.7% in 160 km rides. At the national level, with a larger proportion of non-professional riders, there may be a different set of risk factors for elimination in rides compared to those identified for FEI level rides.

Effect of day, age, sex, and trainer ranking on horse training measured with a GPS recorder

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Training records of racehorses are important to improve training programs to achieve better performance; however, training programs have been measured subjectively and recorded manually. The purpose of this study was to record training programs of racehorses using objective measurements from a GPS recorder and evaluate the effect of day of the week, age, sex, and trainer's ranking. Training programs of 807 racehorses belonging to 42 stables were recorded for about one month using a GPS recorder. Daily distance moved at walk, trot, and jog (≤7 m/s), canter (≤13 m/s), and gallop (>13 m/s) were calculated. Average distance at each velocity for each horse was calculated for each day of the week. The effects of day of the week, sex, age, and trainer's ranking (race wins) on the distance at canter and gallop, and total distance were evaluated by the Steel-Dwass test. The daily median distance at walk, trot, jog, canter, gallop, and in total were 7,884, 864, 535, 1,258, 432 and 11,395 m, respectively. Total distance was longer on Wednesdays and was shorter for females, 2-year-olds, and with lower ranked trainers. The distance at canter was longer on Tuesdays, for 2- and 4-year-olds, and with lower ranked trainers. The distance at gallop was longer on Wednesdays and Sundays, with higher ranked trainers, and shorter for females and 2-year-olds. Trainers adjusted the training strength according to sex and age, and higher ranked trainers galloped longer and cantered shorter during training.

Swimming exercise as part of the training regimens for Thoroughbred racehorses in Australia

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Swimming can be used as an adjunct to over ground exercise in Thoroughbred racehorses with a variety of potential perceived benefits, however, the extent to which it is employed is unknown. We aimed to investigate swimming practices by interviewing a convenience sample of 96 Victorian Thoroughbred trainers with all license categories represented. Swimming was used by 83.3% of trainers. Reasons (and approximate trainer percentages) included 'mental freshness' or variety (60%); to provide small additional fitness benefits (50%); to replace track work particularly on slow days (40%); pre-track work if prone to myositis (30%); 'cool down' after work (20%); to manage body condition of heavy horses/colts (30%); replace some track work for horses with leg problems (10%); and maintain fitness for brief periods when track work was contraindicated (10%). Reasons for not swimming included trainer factors (lack of access to facilities, inexperience in swimming as a training modality) and horse factors (demeanour or distress, history of swim colic and risk of drowning), but few considered EIPH or back problems to be a contraindication. Protocols varied widely particularly in respect to location (shared pool, private facility, beach), frequency (range from <1/week to twice daily), distance/time (range 0.5-10 min), whether continuous or as intervals, whether pre- or post-track exercise and depending on season and stage of training and race distance. Trainer opinions on benefits or contraindications and protocols vary widely with respect to swimming exercise in horses. The role of swimming exercise requires further study so that evidence based recommendations can be made.

Effects of boots on heart rate and respiratory rate in Icelandic horses at trot on a treadmill

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In Icelandic horses the maximum weight of boots allowed in competition is 250 g. Heart rate (HR) and respiratory rate (RR) are often used to estimate exercise intensity of horses. The aim of this study was to determine if 240 g boots had an effect on HR and RR in Icelandic horses at trot. The hypothesis was that boots would increase HR and RR compared to no boots. The study had a crossover design with eight horses and two treatments (no boots or 240 g boots) and was performed on two days on a high-speed treadmill. All horses were accustomed to treadmill exercise. Horses performed a standardized warmup (5 min walk at 1.6 m/s, and 5 min trot at 4.5 m/s), and an exercise test (10 min at trot, 4.5 m/s) with 1 min at walk (1.6 m/s) at the end. HR was measured with Polar RS800CX during the exercise test and RR was counted immediately afterwards. The results were analysed with ANOVA (Proc Mixed) in SAS, including treatment and day as fixed factors and horse as a random factor ($P<0.05$). There was no difference in HR during the exercise test performed with or without boots (127±3 vs 124±3 bpm, respectively; $P=0.185$) or in RR (129±10 vs 123±10 breaths/min, respectively; $P=0.262$). It was concluded that the extra weight of boots did not increase HR and RR. However, further studies regarding boots are needed for exercise of higher workload and on the orthopaedic effects.

Injuries and wastage of Australian Polocrosse horses and survey of training and management practices

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Little information is available on the international sport of Polocrosse. Data relating to training and management practices, injuries during competition and throughout a season for Australian polocrosse horses were collected by the veterinarian at 2014-2016 National Championships and by interview using 76 survey questions with a convenience sample of 54 riders (107 horses), with all rider categories and states represented. At Nationals, 'vet-out' rate was 8.1% (77/951 horses) and was higher for male riders and greater travel distance to competition. The leading causes of 'vet-out' were musculoskeletal injuries (including back-soreness and one catastrophic fracture), then lacerations (7.8%), with one sudden death during play. Mean duration of training before Nationals was 9.7 weeks and protocols varied widely. Of surveyed horses, 30% trained on a similar surface to the tournament field, 21% were stabled at least part-time, and 5% had their own saddle. Use of gastroprotectants (19%), vaccines (<53%) and medications was low. During the surveyed season, 26% of horses had injuries (lacerations; injury to foot, joint, eyes; and pastern fractures) of which 35% required >3 months rest. Lacerations were the most common reason for rest <3 months and tendon injuries for rest >3 months. Training protocols may not adequately prepare horses for competition. Back-soreness and sharing of saddles was common and investigation of causes of back-soreness appears warranted. Further research is needed to evaluate rider category, saddle-fit, distance travelled and preparation for competition as potential risk factors for injury and to make recommendations for managing horses to reduce injury risk.

Individual variability in adaptation to normoxic and hypoxic training in Thoroughbred horses

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The purpose of this study was to examine adaptive responses in horses to normoxic and hypoxic training and to identify responders and non-responders to hypoxic training. Eight untrained Thoroughbred horses (6.5±1.7 years) completed 4 weeks (3 days/week) of two training protocols, consisting of 2 minutes at 100% of maximal oxygen consumption (VO_{2max}) in hypoxia (HT; F_IO_2=15.0%) and in normoxia (NT; F_IO_2=20.9%) in a randomized crossover study with a 4-month washout period. Normoxic incremental treadmill tests were conducted before and after training. Blood gas variables were measured during exercise in the first week of training. Effects of training protocols and periods were analysed using mixed models, and Pearson correlation and a Benjamini-Hochberg procedure were used to evaluate relationships between variables. Training responses to HT were larger (P<0.05) than those to NT in run time (+126±48 vs +49±28 s), VO_{2max} (+18±15 vs +4±11 ml/kg/min) and cardiac output (+1.12±1.21 vs -0.20±0.75 ml/kg/s). There were no effects of periods and no correlations between HT and NT for individual changes in any parameters after training. Arterial O_2 saturation during exercise in HT was positively correlated with changes in run time (r=0.85), cardiac output (r=0.80) and stroke volume (r=0.86) of HT (P<0.05), whereas, no correlations were observed in NT. These results suggest that the individual response to HT and NT is highly variable and the training response within a given horse after NT cannot predict the response after HT (and *vice versa*). However, the severity of hypoxemia during hypoxic exercise might enable prediction of responders to HT.

Sensor based training registration in riding horses – a pilot study

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To increase understanding of training impact on injury development and performance in riding horses, quantitative registration of training is beneficial. A pilot study was performed registering training from 24 riders of 29 horses for 8 weeks. Inertial measurement units (Equisense) were used attached to the girth of the saddle and connected to a smartphone application. The sensor detected gaits and quantified time in each gait, validated internally (>100 horses) by Equisense. Horses were used for either dressage (n=15), show jumping (n=5) or all-round riding (n=9). Effect of discipline purpose on training composition was analysed by a mixed model including repeated effect of horse and fixed effects of week and stable. A normal training week (n=139) included a mean of 5±1 training sessions, with no difference between disciplines. A mean training session (n=663) lasted for 42±9 minutes, including 25±8 minutes of walk, 11±4 minutes of trot and 7±3 minutes of canter. Discipline affected gait distribution within session where horses used for dressage trotted more (12±1 minutes) than the all-round horses (9±1 minutes, $P<0.01$), and cantered less (5±1 minutes) than both the show jumping horses (8±1 minutes, $P<0.05$) and the all-round horses (8±1 minutes, $P<0.01$). The variation in training regimes between purposes of the horses indicate that results from a larger group could possibly provide information on correlation between training session composition and risk of injury. However, for accurate registrations, a reliable (preferably independently validated) and user-friendly device as well as good rider compliance is crucial.

Relationship between speed, plasma lactate concentration and judgement scores in Icelandic horses

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In an official breed evaluation field test (BEFT) for Icelandic horses, all gaits, spirit, and form under rider are scored from 5 to 10 by judges. The aim of the study was to determine any relationship between speed during BEFT, plasma lactate concentration (Lac) after the BEFT and judgement scores. The hypothesis was that speed and Lac were correlated to some of the judgement scores. Data were collected from 266 horses (age 4-11 years) shown at a BEFT in Iceland in 2011. Horses were ridden on a straight track. Venous blood samples were taken within 5 min after the riding assessment, for analysis of Lac. Velocity during the riding assessment was recorded with GPS (Polar, Kempele, Finland). Judgement scores were obtained from the international database Worldfengur. Correlations (Proc Corr) were performed in SAS ($P<0.05$). Total score for riding abilities and the score for pace were positively correlated with Lac ($r^2=0.07$ and $r^2=0.13$, respectively, $P<0.001$). Total score for riding abilities was correlated with average speed during the BEFT ($r^2=0.05$, $P<0.001$) and peak speed during BEFT ($r^2=0.06$, $P<0.001$). The score for gallop and that for spirit were also positively correlated with peak speed during the BEFT ($r^2=0.05$ and $r^2=0.04$, respectively, $P<0.001$). The correlations were weak, but together they show that fast horses received higher scores, which fits with the judging scale. The correlations between Lac (mean±SD: 18.0±6.5 mmol/l) and BEFT scores indicate that anaerobic metabolism had an important contribution to performance of Icelandic horses showed in BEFT.

Effect of high-intensity conditioning and 2 races on aerobic and anaerobic capacities in racehorses

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The objective was to assess the impact of high-intensity racetrack conditioning on aerobic and anaerobic capacities in Thoroughbreds in a prospective, randomised, controlled study. The effect of eight weeks race conditioning and two simulated races on VO_{2max} and maximum accumulated oxygen deficit (MAOD) were evaluated. An incremental treadmill test to determine VO_{2max}, followed by three supramaximal runs to fatigue (105%, 115% and 125% of VO_{2max} in randomized order) were performed at each timepoint (T1 [pre-conditioning] and T2 [post-conditioning]). Racehorses (n=8) were de-trained for 4-6 weeks prior to T1 after an 11-12 month season. Paired variables between T1 and T2 were analysed using a paired t-test, and 2-way RM ANOVA followed by a Holm-Sidak post-hoc compared variables with >1 measurement at T1/T2 ($P \leq 0.05$). VO_{2max} speed ($P=0.04$) and VO_{2max} ($P=0.01$) increased with conditioning. Speeds increased for the 105% ($P=0.02$) and 115% ($P=0.03$) runs but not for 125% VO_{2max} ($P=0.08$). There was no conditioning effect on time to fatigue ($P=0.34$), although it was different between all intensities (105 vs 125% $P=0.001$; 105 vs 115% $P=0.03$; 115 vs 125% $P=0.03$). O_2 demand increased with conditioning ($P=0.02$) for each supramaximal intensity. On average, horses' aerobic capacity improved 4.43%. MAOD was unchanged with conditioning ($P=0.25$) and unaffected by exercise intensity. The relatively small increase in VO_{2max}, with no conditioning-associated change in MAOD contradicted previous reports. However, pre-conditioning MAOD was higher than previously reported and the horses had been very fit. Horses detrain more slowly than humans, so insufficient detraining time could explain the results.

Physiological response to training as indicator of fitness and performance potential in event horses

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Systematic fitness monitoring in eventers is rare as the implementation of standardized tests is commonly perceived as disturbance of the usual training routine. Validity of exercise testing under training conditions should therefore be investigated. Velocity (V) and heart rate (HR), recorded by a GPS and Polar HR monitor throughout 202 hill gallop exercises and blood lactate concentration, measured immediately post-exercise (LAC_p) in 33 1*-4* level eventers were analysed. Linear regression of HR, averaged over predefined speed ranges, was used to calculate V at HRs of 150 (V_{150}) and 200(V_{200}) beats/min. A single exponential regression model, fitted to LAC_p in relation to HR values from all horses, was used to predict LAC_p for each horses' individual HR during exercise. The difference between measured and predicted LAC_p (LAC_{diff}) was then calculated. HR after 1, 2 and 3 minutes of recovery, expressed as percentage of peak HR at cessation of exercise, was derived from bi-exponential regression of HR during the first 3 minutes of recovery after exercise. V_{150} and V_{200} significantly increased with progressing training (paired t-test; $P<0.05$). Competition performance was positively correlated with V_{150} and V_{200} and negatively correlated with LAC_{diff} and recovery HRs (rank correlation; $P<0.05$). Horses with superior competition performance had higher V_{150} and V_{200} values, lower LAC_{diff} values and lower recovery HRs (t-test; $P<0.05$). HR and LAC_p measurements during gallop exercises provided valuable measures of training progress and had predictive value for competition success. This approach may contribute to an increased applicability of field performance monitoring in eventers.

Relative aerobic energy contribution in endurance horses and Thoroughbreds during strenuous exercise

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The objective was to compare Arabian endurance and Thoroughbred racehorses' responses to a maximal intensity standardized incremental treadmill test (MaxSIT) with respect to: (1) their relative aerobic contributions during maximal exercise and (2) selected physiological parameters. Six high-level endurance Arabians and six race-ready Thoroughbreds performed a maxSIT with 1 min increments starting at 8 m/s and increasing until maximum heart rate (HR_{max}) was reached. Measured parameters were HR, blood lactate (BL), haematocrit (Hct), minute ventilation (VE) and oxygen consumption VO_2. Calculated parameters were VO_{2max}, $\%VO_{2max}$ at 10 m/s ($\%VO_{2max}$10 m/s), V_{200}, V_{160}, VLa_4, lactate at HR_{200} (BLa_{200}), and Hct_{max}. The relative aerobic energy input was calculated using the $\Delta BL_{Peak\text{-}Resting}$ increase as previously described. Data were expressed as median±SD and analysed with a Wilcoxon rank sum test ($P<0.05$). There was no difference in duration of the maxSIT between endurance and Thoroughbred horses. Endurance horses had greater VO_{2max} (202.5±14.5 vs 148.2±3.4 ml/kg/min; $P<0.001$) and a greater aerobic contribution at $100\%VO_{2max}$ exercise intensity (89.9±4.6 vs 82.1±4.6%; $P=0.009$) than Thoroughbreds. Endurance horses had a lower HR_{max} (211±7.1 vs 225±4.8 bpm; $P=0.008$), BLa_{200} (3.7±1.4 vs 5.0±1.8 mmol/l; $P=0.004$) and Hct_{max} (56.4±2.3 vs 62.8±4%; $P=0.002$). Endurance horses had VE=1,683±221 l/min; VLa_4=11.7±1.4 m/s; and V_{200}=11.9±0.9 m/s. Because of the HR and speed characteristics of modern endurance races, new proposed parameters calculated for endurance horses were V_{160}=8.5±0.8 m/s and $\%VO_{2max}$10 m/s=73±4%. Trained endurance horses have a greater VO_{2max} and relative aerobic contribution to their energy needs at maximal intensity exercise despite a lower blood oxygen carrying capacity. They produced less lactate during maxSIT.

Nutrition

Effects of vitamin E dose and form on blood parameters in exercising horses

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This study investigated various effects in exercising horses of (1) supplemental vitamin E above NRC recommendations and (2) natural vs synthetic vitamin E. Following a 14 d washout period on a control diet (C: no added vitamin E), 18 horses were divided into three treatment groups and fed C plus (1), 1000 IU/d synthetic α-tocopherol acetate (SYN-L), (2) 4,000 IU/d synthetic α-tocopherol acetate (SYN-H), or (3) 4,000 IU/d micellized RRR-α-tocopherol (NAT). After a 7 d acclimation, standard exercise tests (SET) were undertaken before and after a 6-week exercise protocol. Resting, pre-feeding venous blood samples were collected on days 0, 7, 29, 49, pre- and 2 h post-SET, and 24 h post-SET2. Data were analysed using SAS version 9.4 proc mixed for repeated measures over time with time and treatment as variables. No differences were observed between treatment groups in serum α-tocopherol at d0 and pre-SET1. At all other times, NAT horses had higher serum α -tocopherol concentrations ($P<0.05$). Plasma malondialdehyde concentration was lower in NAT vs SYN-L horses post-SET2 ($P=0.02$). Serum aspartate aminotransferase (AST) was significantly lower post-SET2 in NAT horses vs SYN-L or SYN-H ($P<0.05$) but there was no effect on creatine kinase activities. No significant advantage of SYN-H was found. Micellized RRR- α -tocopherol appeared superior to synthetic all-rac-α-tocopherol in raising and maintaining serum α-tocopherol concentrations, most likely reflecting the higher content of available α-tocopherol. This in turn was associated with lower concentrations of AST and malondialdehyde post-exercise following six weeks of conditioning, which may reflect reduced exercise-induced oxidative stress.

Effects of DHA supplementation on cardiac response in trotters: a double-blind controlled study

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In humans, many researchers have hypothesized that omega-3 fatty acid supplementation would provide benefits during endurance exercise by improving cardiac function or modulating oxygen consumption. The aim of the study was to evaluate the effects of a 90-day oral supplementation with DHA on cardiac response in trotters under training. Thirty-three healthy 3-year-old trotters were randomly assigned to a supplemented group (20 g algal DHA/day), or to a control group. Before and after the 90-day supplementation, plasma DHA concentration was evaluated by HPLC. At rest, mean heart rate and heart rate variability (HRV) were assessed. On the track, speed, heart rate and lactates were evaluated during standardized exercise test, allowing calculation of V200 and VLa4. The DHA effect was assessed by repeated measures analysis of variance. Data were analysed on 15 and 10 horses for DHA and control groups, respectively. Mean plasma DHA concentrations increased only in the DHA group. At rest, mean HR remained constant in the DHA group whereas it increased in the control group and a trend for a higher HRV was observed in the DHA group. During exercise, the increase in V200 was significantly higher in the DHA group as compared to the control group, whereas the change in Vla4 was the same in both groups. The investigated DHA supplementation induced significant changes in HR at rest and exercise without affecting lactate metabolism. These findings are consistent with many observations in athletes and rodents.

Effect of complexed trace mineral supplementation on joint health in young, exercising horses

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To test the hypothesis that complexed trace minerals (CTM) would benefit articular cartilage, 16 Quarter Horse yearlings (9.1±0.17 mo) entering a submaximal exercise training program (15 min walk, 10 min trot, 5 min canter/d; 5 d/wk) were balanced by age, sex, and BW, and randomly assigned to either CTM (2.9, 4.1, and 0.8 mg/kg BW/d Zn, Mn, Cu amino acid complexes, respectively, and 0.1 mg/kg BW/d Co glucoheptonate; n=8) or inorganic (2.0, 3.5, and 0.7 mg/kg BW/d Zn, Mn, Cu sulphates and 0.08 mg/kg BW/d Co carbonate; n=8) microminerals for 12 wk. Respective diets were fed for 12 wk prior to training commencing. Synovial fluid samples were collected at wk 0, 8, and 12 of exercise, and analysed for concentrations of chondroitin sulfate-846 (CS-846), carboxypropeptide of type II collagen (CPII), and collagenase cleavage neoepitope of type II collagen (C2C). Treatment differences were detected using PROC MIXED in SAS (v9.4) with diet, time, and diet-time interaction included as fixed effects and horse(diet) as a random effect. At wk 12, CPII was higher ($P \leq 0.0001$), and C2C ($P < 0.0001$) and CS-846 ($P = 0.005$) were lower than at wk 0. The ratio of CPII:C2C, or synthesis to degradation, increased from wk 0 to 8 ($P < 0.0001$) in all horses but continued increasing to wk 12 ($P = 0.015$) in CTM horses only. Dietary trace mineral source appears to influence cartilage synthesis relative to degradation after 12 wk of low-intensity exercise training in young horses. Complexed trace minerals may improve joint health as a horse progresses through its performance career.

The effects of exercise plus weight loss on insulin sensitivity in obese equids

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This study examined the effect of a practical low-intensity exercise regimen, in addition to dietary restriction, on indices of insulin sensitivity (SI) in obese equids. Twenty-four obese (BCS≥7/9) equines (8 Standardbreds, 8 ponies, 8 Andalusians) were acclimated to a diet of *ad libitum* grass hay for 4 weeks followed by 12 weeks of dietary restriction. Animals were blocked by breed and randomised to one of two groups: dietary restriction only (DIET) or dietary restriction plus exercise (DIET+EX). All animals were provided with a restricted daily ration of grass hay at 1.25% bwt (DM basis) plus 1 g/kg bwt soybean meal and vitamin/mineral powder; 11.5 MJ/100 kg bwt, approximately 85% maintenance DE requirements. The DIET+EX group were exercised on an automated horse walker five days/week; each session consisted of 5 min walk, 15 min trot (2.0-3.5 m/s, according to size) and 5 min walk. Before and after dietary restriction, total body fat mass (TBFM) was determined by deuterium oxide dilution and indices of SI were calculated using minimal model analysis of an intravenous glucose tolerance test. Data were analysed using a mixed-model ANOVA for repeated measures. TBFM was significantly reduced in both DIET and DIET+EX groups after dietary restriction ($P<0.05$). SI was not detectably different in the DIET group after dietary restriction ($P>0.05$), but was higher in the DIET+EX group compared with baseline values ($P<0.05$). These data suggest an additional beneficial effect of low-intensity exercise on insulin sensitivity in obese equines subjected to dietary restriction to achieve weight loss.

A comparison of diet and exercise for weight loss in obese horses

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This study was designed to determine if exercise (EX) or diet (DIET) is more effective to achieve weight loss and improve glucose metabolism in horses. The study consisted of a 2-week adaptation period (offered 100% DE requirements), followed by a 4-week treatment period. Ten obese horses were paired according to gender, age, and breed. One horse from each pair was assigned to either DIET (n=5; offered ~83% DE requirements) or EX (n=5; net ~83% DE requirements; offered 100% DE and exercised using an automatic exerciser to expend ~17% weekly DE based on heart rate). Diets consisted of mixed grass hay, salt block, and a vitamin/mineral supplement mixed with ~260 g of beet pulp. Chemical analysis was used to determine feed DE values. Body weight (BW) was measured weekly, and DE values were adjusted accordingly. An oral glucose test was administered on days 0 and 28. Treatment effects were tested using repeated mixed measures. Insulin to glucose ratio (Ins:glc) data were not normally distributed, and were log-transformed for analysis. The EX and DIET groups both lost significant amounts of BW (5.4±1.8% and 5.8±1.3% BW, respectively), with no difference between the groups. However, Ins:glc responded differently over time, with the EX group showing a significant decrease from d0 to d28 (0.32±0.17 to 0.19±0.08 mU/l Ins:mg/dl glc, respectively), while the DIET group showed no change. While both exercise and diet were effective weight loss strategies for horses, only exercised horses showed improvements in glucose metabolism.

Improved performance indices in horses following restricted diet

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Several studies have indicated a negative relationship between body fat content and performance in horses but this has been minimally studied within individuals. We studied the effect of reduced body weight (BW) and body condition score (BCS) on plasma lactate and glucose responses to exercise in nine Icelandic horses. A crossover design was used (two treatments × 5 weeks) and differences in body condition were induced by a 5-week period with restricted feed allowance (RD). Horses were fed grass haylage either to maintain/gain BW (HD: 17 MJ metabolizable energy [ME]/100 kg body weight [BW]) or to lose BW (RD: 8.5 MJ ME/100 kg BW) but were trained similarly. During the last week, an incremental exercise test was performed on a treadmill (four 2-min steps, 6.25% incline) and BW and BCS (scale 1-9) were registered. Data were analysed using ANOVA (proc GLM) and a model including horse, period and treatment. Horses were heavier on HD than on RD (406±1 vs 389±1 kg, $P<0.0001$) and had higher BCS (6.5±0.0 vs 6.2±0.0, $P<0.0001$). Mean plasma glucose concentration during exercise was higher on RD than on HD (5.3±0.1 vs 4.8±0.1 mmol/l). The fourth step plasma lactate concentration was lower in RD than in HD horses (5.6±0.2 vs 6.5±0.2 mmol/l, $P<0.05$) and VLa4 decreased by 0.3 m/s in HD ($P<0.05$). It was concluded that restricting the diet improved performance and that further studies are needed to understand the multifactorial mechanisms behind this.

A marine-derived calcium supplement increases bone density in Thoroughbred racehorses

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Bone density and strength are important for the long-term health and soundness of performance horses. Marine-derived calcium mineral complexes have been shown to improve bone density and strength in laboratory animals and humans. This study evaluated the effect of a supplement containing marine-derived calcium (Triacton™) (TA) on bone density in Thoroughbred racehorses. Thirteen horses (average age=3.1±0.4 y) were used in a 12-week training study. Horses were fed a timothy hay and fortified concentrate ration which supplied 64 g Ca and 42 g P/d. Seven horses received 120 g/d of TA which supplied an additional 15 g Ca and 7 horses received 120 g/day of a placebo pellet (CON). Training consisted of jogging, galloping and breezing. There was no difference in training intensity or duration between the two treatment groups. Radiographs of the left front cannon bone were taken from a dorsal-palmar and a lateral-medial view at 0, 4, 8 and 12 weeks of the study. An aluminium step-wedge was placed in plane with the cannon bone to use as an external measure of bone density. Radiographic photodensitometry was used to measure the density of the lateral, medial, dorsal and palmar cortices of the cannon bone. Differences due to training duration and supplementation were determined using a 2-way ANOVA. Over the 12-week training period the horses supplemented with TA increased dorsal and palmar cortical bone density ($P<0.05$). CON densities were not affected by training. Medial and lateral cortical bone densities were not affected by treatment.

Overfeeding may affect locomotion symmetry

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Excessive body weight (BW) and fat content may increase workload and impair performance. Possible impact of greater BW and condition on locomotion symmetry were investigated in a 2×5 week crossover study involving nine Icelandic horses in training. Horses were fed either to maintain/gain (HD: 17 MJ metabolizable energy [ME]/100 kg BW) or lose (RD: 8.5 MJ ME/100 kg BW) BW but were trained similarly. At the end of each period, BW and body condition scores (BCS, scale 1-9) were registered and locomotion symmetry was assessed before, one and three days after a ridden exercise test (11 min, peak HR 217 bpm) with a 3-inertial sensor system at trot in a straight line by hand on one hard and one soft surface. Vector sums (VS) of head and pelvic min and max differences were calculated for front and hind limbs, respectively. After treatment HD, horses were heavier (406±1 vs 389±1 kg, $P<0.0001$) with higher BCS (6.5±0.0 vs 6.2±0.0, $P<0.0001$) than after RD. Vector sums were analysed using a mixed model including repeated effect of individual and fixed effects of diet, surface, day and period. VS front were higher on HD than on RD (11.2±1.2 vs 9.0±1.1, $P<0.01$), but there was no effect of treatment on VS hind (HD: 5.7±0.5 vs RD: 4.9±0.5, $P>0.05$). It was concluded that greater body weight and/or fat deposition might have a negative impact on locomotion symmetry. Further studies on the effect of BW and body condition on long-term orthopaedic health are needed.

The effect of moderate-intensity exercise on voluntary intake and glucose metabolism in horses

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This study aimed to determine if exercise increased feed intake. Eight horses underwent an 8-d adaptation period to a diet of *ad libitum* grass hay, a 7-d baseline period in which baseline dry matter intake (DMI) was quantified, and a 3-wk treatment period. Horses were assigned to exercise (EX, n=4; exercised on an automatic exerciser to expend ~15% of DE requirements based on heart rate) or control (CON, n=4; no forced exercise). Treatment size was determined using a power calculation with data from a previous study, with an additional animal added to each group. Body weight (BW) was monitored weekly. An oral glucose test was administered at the beginning and end of the treatment period. Treatment effects were examined using a repeated mixed measures design (using baseline DMI as a covariate for DMI analysis). Insulin to glucose ratio (Ins:glc) data were not normally distributed, and were log-transformed. The EX and CON groups had similar DMI (2.21±0.22% and 2.09±0.25%, respectively), relative to their baseline DMI. BW responded differently over time, with the CON group gaining weight, while the EX group did not change. The EX group also showed a reduction in Ins:glc from the beginning to the end of treatment (0.24±0.14 to 0.16±0.09 mU/l Ins:mg/dl glc), while the CON group showed no change. Overall, exercise did not result in a difference in DMI over a three-week period, but appeared to affect glucose metabolism, even in the absence of weight loss.

The effect of diet composition and exercise on changes in caecal pH and SCFA composition

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The objective of this study was to measure changes in caecal pH and short chained fatty acids (SCFA) composition when horses were fed 4 different diets and exercised at 4 different times after feeding. Caecum cannulated Norwegian cold-blooded trotters (n=4) were fed 4 iso-energetic diets (50% above maintenance): (1) 14.5 kg hay; (2) 7.5 kg hay + 2.2 kg sugar beet pulp + 0.4 kg oil; (3) 6 kg hay + 4.3 kg oats; or (4) 6 kg hay + 3.8 kg barley. Horses were fed three times a day and all diets were supplemented with salt, vitamins and minerals. The horses undertook a standardized sub-maximal exercise test 2, 4, 6 or 8 hours after feeding (on 4 consecutive days) using a high speed treadmill for 40 minutes with variable speeds between 1.5-4.2 m/s, a 3% slope and up to 16 kg pull through lines attached to the breast-band harness. Caecal pH was measured continuously and caecal fluid was sampled hourly for 9 hours and analysed for SCFA. Data were analysed separately for each diet in a model comprised of the fixed effect of exercise, time after feeding, and their interaction. Results showed that caecal pH and differences in SCFA composition were affected by time after feeding, as expected. However, there was also a significant overall effect of exercise on pH, SCFA concentrations and SCFA composition. In conclusion, the results show that not only diet and time after feeding, but also time of exercise in relation to feeding, affect the chemical environment and metabolism in the hindgut of horses.

Complexed trace minerals: antioxidant status and oxidative stress in young exercising horses

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To test effects of dietary trace mineral source on antioxidant status during training, Quarter Horses (9.7±0.7 mo) balanced by gender, age, and BW were assigned to complexed (CTM; 2.9, 4.1, and 0.8 mg/kg BW/d Zn, Mn, and Cu amino acid complexes, respectively, 0.1 mg/kg BW/d Co glucoheptonate, 3.5 mg/kg BW/d CP; n=8) or inorganic (INORG; 2.0, 3.5, and 0.7 mg/kg BW/d Zn, Mn, Cu, respectively, 0.08 mg/kg BW/d Co, 3.4 g/kg BW/d CP; n=8) trace minerals. After 12 wk on dietary treatments, light, standardized exercise training commenced. Whole blood and muscle samples were collected before and after 12 wk of training, 15 hours after the last bout of exercise. Samples were evaluated for antioxidant enzyme activities and lipid peroxidation. Data were analysed using PROC MIXED in SAS 9.4 with diet, training, muscle, and all interactions as fixed effects and horse(diet) as a random effect. Superoxide dismutase (SOD) activity increased from wk 12 to 24 in CTM gluteus medius (GM; P=0.014) and INORG triceps brachii (TB; P=0.049), and tended to increase in CTM TB (P=0.063). Muscle glutathione peroxidase (GPx) activity was higher for CTM than INORG (P=0.001). Blood SOD and GPx activities were unaffected by diet and training. Muscle malondialdehyde tended to decrease from wk 12 to 24 (P=0.067), but was not different between dietary treatments at wk 24. Horses receiving CTM showed increasing GM SOD activity, and higher muscle GPx activity than INORG through 12 wk of exercise training. Complexed trace minerals may increase muscle antioxidant capacity in young performance horses.

The effects of blueberry consumption on reactive oxygen species in sedentary and exercising horses

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Blueberries are high in antioxidants. The purpose of this study was to determine plasma total reactive oxygen species (ROS) in exercising horses fed blueberries. Adult Thoroughbred horses, allocated to five groups, were fed grain (Purina®, Strategy®, Purina Animal Nutrition, Gray Summit, MO, USA) containing 4% blueberries (BB) or no blueberries (C). Groups were (1) sedentary BB (n=6); (2) sedentary C (n=6); (3) sedentary BB 10 days, then exercise BB 10 days (n=5); (4) exercise C 10 days, then exercise BB 10 days (n=5); (5) exercise C 10 days, then exercise C 10 days (n=6). Horses were conditioned on a high-speed treadmill for 8 days between the standard exercise tests (SET) on days 0, 10 and 20. Plasma was obtained from a pre-placed jugular catheter and collected into heparin before and during the SET. Plasma ROS rates were measured using electron paramagnetic resonance spectroscopy. ANOVA was used to detect differences in day, time and their interactions, followed by a post-hoc Tukey's test to determine differences ($P<0.05$). Mean (±SEM) ROS activity increased significantly from 0.6809±0.0400 in BB-fed and 0.7137±0.0383 in control horses on Day 0 to 0.7828±0.0400 and 0.8946±0.0383, respectively, during exercise on Day 10. Mean ROS activity was significantly lower in the BB-fed horses by Day 10. After twenty days of exercise, ROS significantly decreased during the SET in blueberry-fed (0.5835±0.0400) and control (0.3289±0.0381) horses. Blueberry feeding and exercise decreased ROS in exercising horses. Funded by the U.S. Highbush Blueberry Council.

Blueberry feeding improves haematological parameters in exercising horses

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Blueberry feeding delays fatigue in animals and might improve haematologic parameters. The purpose of this study was to evaluate effects of blueberry (BB) feeding on packed cell volume (PCV) and total solids (TS). PCV and TS were measured in horses during a treadmill standardized exercise test (TSET). Adult Thoroughbred horses were allocated to five groups and fed grain (Strategy®, Purina Animal Nutrition, Gray Summit, MO, USA) containing 4% (BB) or no BB (C). Groups were (1) sedentary BB (n=6); (2) sedentary C (n=6); (3) sedentary BB 10 days, then exercise BB 10 days (n=5); (4) exercise C 10 days, then exercise BB 10 days (n=5); (5) exercise C 10 days, then exercise C 10 days (n=6). Horses underwent a TSET on Days 0, 10, and/or 20. PCV and TS were measured by capillary tube method and refractometer, respectively, before and during the exercise. Exercise groups were conditioned for 8 days between TSET on days 10 and 20. An ANOVA was used to detect differences in day, time and their interactions and a post-hoc Tukey's test was used to test significance ($P<0.05$). PCV and TS increased significantly during exercise. By day 20, peak mean [SEM] PCV was significantly lower (45.8% [2.1]) in BB-fed horses, compared to C horses (50.5% [2.8]). Mean [SEM] TS concentration was lower in the BB-fed horses (7.9 g/dl [0.2]), compared to C (8.1 g/dl [0.2]), but was not significantly different. Blueberry-fed horses had lower hematologic parameters during moderate exercise, which could decrease blood viscosity, improve cardiac output and decrease peripheral vascular resistance. Funded by the U.S. Highbush Blueberry Council.

Blueberry feeding improves volume of work in exercising horses by lowering heart rate and lactate

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Blueberry feeding decreases blood lactate and delays fatigue in animals. The purpose of this study was to measure heart rate (HR) and blood lactate (L) during a treadmill standardized exercise test (TSET) in blueberry (BB) fed horses. Adult Thoroughbred horses were allocated to 5 groups and fed grain (Strategy®, Purina Animal Nutrition, Gray Summit, MO, USA) containing 4% BB or no BB (C). Groups were (1) sedentary BB (n=6); (2) sedentary C (n=6); (3) sedentary BB 10 days, then exercise BB 10 days (n=5); (4) exercise C 10 days, then exercise BB 10 days (n=5); (5) exercise C 10 days, then exercise C 10 days (n=6). Trained horses underwent a TSET. HR was recorded and L was measured (Lactate Plus®, Waltham, MA, USA), before and during the TSET. Horses were exercised between days 0, 10 and 20. ANOVA was used to detect differences in day, time and their interactions, followed by a post-hoc Tukey's test ($P<0.05$). Data are displayed as mean±SEM. Peak mean blood lactate and HR increased significantly during exercise compared to sedentary horses. Peak mean L (1.16±0.26 mmol/l) was lower during the TSET in BB-fed horses, compared to the C horses (1.87±0.24) by day 20. Peak mean HR was significantly lower in the BB-fed horses (159±5.6 bpm) compared to the C horses (177±5.1) by day 20. Although this TSET was moderate, training and blueberry-feeding allowed horses to perform the same work at lower HR and lower blood lactate, which might delay fatigue. Funded by the U.S. Highbush Blueberry Council.

Complexed trace minerals: antioxidant responses to transportation stress in young equine athletes

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Quarter Horses (9.7±0.7 mo) balanced by age, gender, and BW were assigned to complexed (CTM; 2.9, 4.1, and 0.8 mg/kg BW/d Zn, Mn, and Cu amino acid complexes, respectively, 0.1 mg/kg BW/d Co glucoheptonate, 3.5 mg/kg BW/d CP; n=8) or inorganic (INORG; 2.0, 3.5, and 0.7 mg/kg BW/d Zn, Mn, Cu, respectively, 0.08 mg/kg BW/d Co, 3.4 g/kg BW/d CP; n=8) microminerals to assess effects of mineral source and training on antioxidant responses to 1.5 h of trailer transportation. After 12 wk on dietary treatments, light, standardized exercise training began. At wk 12 and 24, gluteus medius (GM), triceps brachii (TB), and whole blood were collected before, and 1- and 24-h post-trailering; blood was also collected 0- and 6-h post-trailering. Samples were evaluated for antioxidant enzyme activities and lipid peroxidation. Data were analysed using PROC MIXED in SAS 9.4 with diet, training, trailering, muscle, and all interactions as fixed effects and horse(diet) as a random effect. At wk12, trailering increased muscle ($P=0.005$) and blood ($P=0.01$) glutathione peroxidase (GPx) activity, and decreased muscle malondialdehyde ($P=0.002$). At wk12, CTM had higher muscle GPx activity ($P=0.0002$) and tended to have lower muscle superoxide dismutase (SOD) activity ($P=0.06$). At wk24, trailering increased muscle GPx activity ($P=0.02$), but did not affect any other measures. At wk24, CTM had higher muscle GPx activity ($P=0.0005$) and tended to have higher blood SOD activity ($P=0.09$). Greater blood SOD and muscle GPx activities at wk24 suggests supplemental CTM and exercise training increased antioxidant capacity during trailering in young performance horses.

Comparison of equine dietary iron requirements to iron concentrations of 5,837 hay samples

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Athletic horses are often supplemented with Fe in an attempt to improve performance. Others claim excess dietary Fe is causative of horses becoming insulin resistant. According to the 2007 Horse NRC, Fe requirements are 40 mg/kg for all classes of horses other than growing foals, lactating and pregnant mares. Nutrient concentrations from hay samples submitted for analysis in 2017 and for which Fe was measured were obtained from Equi-Analytical in the U.S.A., representing 3,060 grass, 1,193 legume, and 1,584 mixed hay samples. Statistical analysis was performed using Proc MEANS of SAS. Mean Fe in grass hay was 212 (SD=268) mg/kg, with the range being 31 to 3,760 mg/kg, and the median being 135 mg/kg. Mean Fe in legume hay was 446 (SD=498) mg/kg, with the range being 47 to 6,360 mg/kg, and the median being 307 mg/kg. The 2005 NRC suggested a maximum tolerable Fe concentration of 500 mg/kg using data from other species. From all hay samples (n=5,837), 707 contained Fe at or above the suggested tolerable threshold while only 15 contained Fe at less than 40 mg/kg, meaning most of these hays would have supplied Fe in excess. Given the dearth of Thoroughbred racehorses that are insulin resistant, despite Fe supplementation in combination with diets that can easily supply amounts beyond requirements, it seems unlikely excess Fe causes insulin resistance. Data also confirm most diets meet Fe requirements without need for supplementation.

Muscle

Assessment of equine muscle mass by 3-dimensional scanning

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Muscle mass has been difficult to accurately assess in horses yet it is a major determinant of strength and athletic performance. Subjective assessment of photographs, measurement of forearm girth and ultrasonic measurement of a muscle's depth or cross-sectional area have all been used to judge muscle mass. None of these techniques, however, provide an accurate measure of the whole-body musculature. The objectives of the present study were to develop a 3-dimensional technique to measure equine muscle mass and to then determine its accuracy through duplicate scans and comparison to body weight. A handheld Occipital Structure Sensor scanner was connected to an iPad and a systematic means developed to scan the torso and hindquarters. Scans were performed twice within 30 min on 8 horses that had affixed anatomical markers and known body weights. A control object was present in each scan. Using the markers and anatomic points, sector volumes such as torso + hindquarter above stifle, lumbar muscles, hindquarter above hock, left and right sides were calculated using Skanect and Meshmixer and percent error was compared for duplicate scans. Correlation between body weight and body scan volume was high at r^2=0.73. Percent error (SD) of duplicate scans was low (range 1.66±1.53 to 6.35±5.82%). Artificial division into sectors introduced the greatest variability. In conclusion, 3-dimensional scanning technology provides a new accurate means to assess muscle mass in horses and to determine the impact of training or nutritional supplements on muscle development.

Effect of hyperthermia on equine skeletal muscle mitochondrial oxygen consumption

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The skeletal muscle of exercising horses develops pronounced hyperthermia during strenuous or prolonged exercise, with very high tissue temperature associated with muscle fatigue or damage. The purpose of this study was to evaluate the effects of physiologically relevant hyperthermia on equine skeletal muscle mitochondrial function, using *ex vivo* measurement of oxygen consumption to assess the function of different mitochondrial elements. Fresh muscle biopsies from three healthy unfit Thoroughbred geldings were permeabilized to permit diffusion of small molecular weight substrates through the sarcolemma and analysed in a high resolution respirometer at 38 and 44 °C. Oxygen consumption was measured under conditions of non-phosphorylating (leak) respiration, phosphorylating respiration through Complex I and Complex II, and uncoupled respiration. Data for each respiration state were compared for effect of incubation temperature using a paired t-test. Leak respiration was ~3-fold higher at 44 °C compared to 38 °C regardless of electron source (Complex I: 22.88±3.05 vs 8.08±1.92 pmol/s/mg, P=0.002; Complex II: 79.14±23.72 vs 21.43±11.08 pmol/s/mg, P=0.022) and accounted for 14.2% of maximal oxygen consumption at 44 °C vs 5.9% at 38 °C. There was not a significant effect of temperature on maximal uncoupled respiration. These results suggest that skeletal muscle hyperthermia decreases the efficiency of oxidative phosphorylation through increased mitochondrial membrane permeability, thus providing a specific biochemical basis for hyperthermia-induced muscle fatigue.

Pre-exercise oral electrolyte supplementation enhances skeletal muscle ion influx during recovery

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Oral electrolyte supplementation (ES) is widely used to replace exercise-induced sweat losses, however the extent of intracellular uptake is poorly understood. It was hypothesized that ES prior to prolonged moderate intensity exercise would increase skeletal muscle Na^+ and K^+ influx during exercise and recovery. In a randomized crossover design, four conditioned mares were nasogastrically administered radioactive ^{24}Na or ^{42}K in a balanced electrolyte solution (ES) or water (W), 1 h before (Pre) or immediately after (End) exercising at 30% VO_{2max} until voluntary fatigue. Gluteus medius muscle and jugular venous blood were sampled immediately before and after exercise, and at 2 and 4 hours of recovery (Rec), and were analysed for radioactivity using a gamma counter. Differences over time and between groups were assessed by repeated measures ANOVA (P<0.05). Muscle Na^+ influx was highest PreEx and lowest at EndEx in all groups (Pre- vs End-Ex; 18.13±3.3 vs -0.31±3.3 mEq/kg/h) with no difference between treatments, while rates of K^+ influx were highest at EndEx (15.52±3.9 mEq/kg/h). PreEx ES resulted in greater muscle ion influxes during Rec compared to all other treatments, such that influx rates for Na^+ and K^+ were 20.34±7.7 and 2.54±2.1 mEq/kg/h at 24 h Rec, respectively. In contrast, there was a net efflux of muscle K^+ (-3.33±2.1 mEq/kg/h) during Rec in the W alone trial. In conclusion, pre-Ex ES resulted in higher rates of Na^+ and K^+ influx into skeletal muscle during the initial recovery period, compared to Post-Ex ES or W alone.

Effect of dietary supplementation with ubiquinol on muscle concentrations of CoQ10

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Coenzyme Q10 (CoQ10) is essential for mitochondrial aerobic production of ATP via oxidative phosphorylation, but has had minimal study in horses. Its biologically active form is ubiquinol. The effects of daily supplementation with ubiquinol on gluteal muscle CoQ10 concentrations and citrate synthase (CS) activities in fit Thoroughbreds were evaluated. CS is an important Kreb's cycle enzyme and an indicator of oxidative phosphorylation status. In this randomized controlled crossover study, 6 horses received either ubiquinol 1 g daily for 3 wks followed by 21 d without supplement, or had a 3 wk unsupplemented period followed by 3 wks supplementation. Middle gluteal muscle biopsies were obtained before feeding on day 0 (baseline), and after 10 d and 21 d of each period. Muscle [CoQ10] was determined by HPLC with UV detection at 275 nm. CS was measured spectrophotometrically at 37 °C and related to mitochondrial [CoQ10]. Results (mean±SD) were analysed by 2-way RM ANOVA for effects of supplementation and time ($P<0.05$). Muscle baseline and non-supplemented [CoQ10] were unchanged (466±197 pmol/mg). Values increased from 413±276 (baseline) to 558±158 pmol/mg after 10 d supplementation ($P=0.03$), but not thereafter (21 d: 696±534 pmol/mg; $P=0.31$). CS activity increased in concert with [CoQ10] ($P=0.02$; baseline: 67±18, 10 d: 155±68, 21 d: 163±78 nmol/(min.mg)). Muscle [CoQ10] was moderately correlated with [CS] ($P=0.002$; $r^2=0.35$). Discontinued supplementation decreased muscle [CoQ10] and approached significance ($P=0.06$). Daily dietary supplementation with 1 g ubiquinol increased gluteal muscle [CoQ10] from d0 to d10, but not from 10-21 d, possibly indicating saturation of mitochondria with ubiquinol. Associated increases in CS activity suggested aerobic metabolic capacity was enhanced with supplementation. Discontinuing supplementation decreased [CoQ10].

Incidence and description of exertional rhabdomyolysis in endurance horses at Tom Quilty Cup 2017

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This study documented the incidence and underlying mechanisms of exertional rhabdomyolysis (ER) at the 2017 Tom Quilty Cup 160 km endurance ride in a cool environment. Data collection included a questionnaire, clinical examination and blood sampling from all hospitalised horses (HH) and 12 non-metabolically compromised horses (NH; finishers n=9, lame n=3). Of the 164 entries, 106 (65%) horses completed the ride. Reasons for non-completion included lameness (n=39; 24%), metabolic disorders (n=8; 5%) and voluntary withdrawal (n=11; 7%). The ambient temperature ranged from 6.4-10.6 °C and RH 92-97%. Mean speed of finishers was 10.1±1.99 km/h. Ten (6.1%) horses were hospitalised including 6 (3.7%) horses with ER (3 Arabians, 3 part-Arabians; mean age 12.8 years) which were eliminated or withdrawn between 60-100 km. All but one travelled from interstate. Clinical signs included a tired appearance, pain/stiffness or lameness. Serum CK and AST activities in ER cases (at hospital entry, range 4-21 h post-start; median 16,021 IU/l [range: 2,149-157,440], and 2,365 [735-6,976], respectively) were significantly different to NH (median 561 [173-3,580] and 385 [213-1,149] IU/l, respectively). Three ER horses had serum CK exceeding 10,000 IU/l. Mean serum electrolytes, lactate and creatinine concentrations were within normal limits, while urea concentration was mildly elevated in HH only (mean±SD, 8.4±2.5 mmol/l). The incidence of myopathy was similar to that reported in horses completing 80 km rides, and serum CK and AST activities were variable. Further research is needed to investigate the possible effects of long distance travel and muscle fatigue on ER development during endurance competition.

Active myeloperoxidase reaches mitochondria of myoblasts and interferes with mitochondrial function

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High concentrations of myeloperoxidase (MPO) were measured in horse muscles after intense exercise, suggesting a possible muscular infiltration of PMNs and/or MPO. In some endurance horses, a severe increase of muscle MPO activity was associated with a large decrease of mitochondrial complex I activity and a high plasma CK activity, demonstrating a possible link between MPO activity and mitochondrial dysfunction. In myoblasts, we showed that MPO amplify the mitochondrial dysfunction during an anoxia/reoxygenation (A/R) phenomenon. The goal of the study was to see whether active MPO added during the myoblast culture affects mitochondrial respiration without induction of an A/R process and reaches mitochondria. Primary equine myoblasts were incubated 2 h with equine MPO (250 ng/ml), Control was made with non-treated cells. The mitochondrial function was assessed by high resolution respirometry. Myoblast fractions (debris/nuclei and mitochondria) were separated by potter homogenizer and differential centrifugations. MPO activity was specifically measured in fractions by SIEFED and reported by the protein content. Assays were made on five independent experiments. The incubation of myoblasts with MPO induced a significant decrease ($P<0.05$) of routine (23%) and maximal (31%) respirations in comparison to control cells. The active MPO content (ng/mg protein) in mitochondrial fraction of MPO treated cells was 14.1× higher than in the debris/nuclei fraction. Results evidence that active MPO can easily reach the mitochondrial compartment leading to a decrease of mitochondrial respiration. Since the MPO remains active in mitochondria, questions are raised about its role on the oxidation of Oxphos complexes and muscle recovery.

Applied physiology – Other (genomics, metabolomics, proteomics, etc.)

Identification of expression quantitative trait loci (eQTL) in the skeletal muscle of Thoroughbreds

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Expression quantitative trait loci (eQTL) are genetic loci associated with variation in gene expression. Variation in gene expression is highly heritable and has been proposed to be a major determinant of phenotype in humans. Considering the complexity of the genetic architecture underlying the athletic phenotype in Thoroughbreds, we hypothesised that eQTL may be identified that contribute to variation in equine skeletal muscle function. Middle gluteal biopsies were collected from untrained 2 year old Thoroughbreds at rest (R, n=92) and 4 hours post-exercise (E, n=77). eQTL were identified by analysing the expression of 13,384 genes, quantified using RNA-seq, and tested for expression association with 26,041 SNP genotypes, generated from equine SNP genotyping arrays, using a linear model (covariates: sex, age and batch). *clusterProfiler* was used to determine functional enrichment of genes among significant eQTL (Benjamini-Hochberg *P*-value<0.05). eQTL were identified for 2,197 genes in R and 2,044 in E cohorts. There was a significant enrichment of genes involved in cofactor and coenzyme metabolic processes among putative cis regulated genes -eQTL within 1 Mb of the gene they were associated with. These included genes with functions related to mitochondrial function, haematopoiesis and nutrient binding (such as selenium). This is the first report of genome-wide eQTL in equine skeletal muscle, which demonstrates heritable variation in the expression of genes relevant to substrate binding and metabolic function. This variation may have implications for aerobic capacity, athletic performance and individual nutrient requirements and may be exploited in the future to individualise nutritional and exercise regimes.

Proteome and transcriptome profiling of equine myofibrillar myopathy

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Equine myofibrillar myopathy (MFM) causes exertional muscle pain and is characterized by myofibrillar disarray and ectopic protein aggregates of unknown origin. To investigate the pathophysiology of MFM, we compared the skeletal muscle proteome and 3 h post-exercise transcriptome of gluteal muscle in MFM and control Arabian horses using iTRAQ and RNA-sequencing analyses. Differential expression (DE) was evaluated using edgeR and pathway analysis using Cytoscape and Cluego. Bonferroni correction was applied to significance set at $P<0.05$. Proteome analysis revealed significantly lower antioxidant peroxiredoxin 6 content (PRDX6), sarcomere protein tropomyosin (TPM2) and higher fatty acid transport enzyme carnitine palmitoyl transferase (CPT1B) in MFM vs control muscle at rest. Three hours after exercise, 191 genes were DE in MFM vs control muscle with a remarkably focused change in genes involved in sulphur compound/cysteine metabolism such as cystathionine-beta-synthase [*CBS*] and a cysteine and neutral amino acid membrane transporter [*SLC7A10*]. In MFM vs control at rest, 284 genes were DE in pathways for structure morphogenesis, fibre organization, tissue development and cell differentiation including >2 log2 fold change in cardiac alpha actin [*ACTC1*] and cytoskeletal desmoplakin [*DSP*]. In conclusion, myofibrillar disarray and protein aggregation in MFM horses was embodied by DE expression in pathways of structure/fibre organization and tissue regeneration. Reduced antioxidant capacity as a potential aetiology for MFM was supported by diminished cysteine rich antioxidant peroxiredoxin 6 with compensatory increased expression of genes involved in cysteine synthesis following exercise.

Signatures of genetic selection for elite performance in the Australian Thoroughbred

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The Thoroughbred traces its ancestry to a small number of foundation mares and stallions bred for racing in England. Horseracing is now a global sport with major hubs in Europe, North America, Australasia and Japan. While there is relatively low genetic diversity in the Thoroughbred, local environmental pressures and regional nuances of racing have led to phenotypic variation across regions. The Hong Kong Jockey Club found that continent of origin was significantly associated with different musculoskeletal conditions. As all Thoroughbreds raced in Hong Kong are imported this suggests there may be region specific genetic and early life management differences. We tested the hypothesis that genes selected for regional phenotypic variation may be identified by analysis of selection signatures in SNP genotype data. Elite Thoroughbreds were genotyped for 48,000 SNPs using commercial genotyping platforms. The Composite Selection Signals method captured highly differentiated loci with excess haplotype homozygosity in the target population by comparing Australian (n=50) vs non-Australian (n=50) horses. Ensembl BioMart identified genes within ±1.0 Mb of selection peaks. A single ~36 kb locus on ECA14 was identified among the top 0.1% SNPs that contained 18 protocadherin genes and *HARS2*. Protocadherins are involved in neural circuit formation and are epigenetically modified in response to early life experiences. *HARS2* is a mitochondrial protein synthesis gene, which is differentially expressed in muscle following exercise and training. This indicates there is differential selection for elite performance under different racing and management conditions. This information may be used to identify Thoroughbreds best suited to race in Australia.

Myostatin genotype specific gene expression in Thoroughbred skeletal muscle

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Myostatin gene (*MSTN*) variation influences distance aptitude in Thoroughbreds as a consequence of functional physiological effects including skeletal muscle fibre type and muscle hypertrophy variation. A promotor region SINE insertion, tagged by SNP g.66493737-C, alters *MSTN* mRNA expression. We tested the hypothesis that skeletal muscle gene expression varies among *MSTN* genotypes as a consequence of differential up- or down-stream gene signalling pathways, which may be influenced by exercise and training and consequently contributes to variation in exercise phenotypes. Middle gluteal skeletal muscle biopsies were collected from n=35 C:C, n=50 C:T and n=9 T:T Thoroughbreds untrained at rest (UR) and 4 hours after exercise (UE), and trained at rest (TR). Gene differential expression (DE) was determined from RNAseq data using *DESeq2* (Benjamini-Hochberg *P*-value <0.05). Functional over-representation analysis was performed in DAVID. In UR samples DE was observed for one, nine and 47 genes between C:C and C:T, C:T and T:T and C:C and T:T, respectively. The *OSGEPL1* gene was DE among all cohorts. Six genes were DE in UE between C:C and T:T including *OSGEPL1*, *FGF10* and *COQ8A*. There was significant enrichment for GO categories related to mitochondria in TR. Comparison of the exercise response (UR vs UE) revealed patterns of expression that were opposing; i.e. *CHRNG* was -1.8-fold in T:T but +>4-fold in C:C. Genes involved in mitochondrial function and located in proximity to *MSTN* were most significantly different among genotype cohorts. Patterns of DE among genotypes suggests gene-regulated influence on the phenotype, and this understanding may assist genotype-guided training strategies.

Analysis of genetic contributions to speed highlight genomic regions responsive to exercise stimuli

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Despite strong selection for athletic traits in Thoroughbred horses, there is distinct variation in speed and aptitude for racing performance. Using global positioning system monitoring during exercise training, we measured speed variables and temporal changes in speed to derive phenotypes to test the hypothesis, using genome wide association studies (GWAS), that genetic variation contributes to speed. Speed was measured during sprint exercise bouts and six key speed indices were derived. Principal component analysis captured 73.9% of the variation in these indices with principal component 1 (PC1). GWAS were performed for median PC1 in two-year-old horses (PC1_2yo, n=122, >3 recordings, <3yo) and temporal change in PC1 (ΔPC1, n=168, >3 recordings over >3 months). PC1_2yo was found to be significantly different among elite (placed at Listed or Group level), non-elite (raced, not placed at Listed or Group level) and unraced horses (ANOVA, *P*<0.02). A GWAS for PC1_2yo revealed a 1.6 Mb candidate region on ECA8 containing the *MYO18B* gene. GWAS for ΔPC1 identified a candidate 1.9 Mb region on ECA11, containing several genes that are significantly differentially expressed in equine skeletal muscle in response to acute exercise and training stimuli, including *MYO18A*. *MYO18A* has been identified as a key regulator of the skeletal muscle response to exercise. The identified genomic variation proximal to the myosin family genes may be important for the regulation of the response to exercise and training. Genomic variation in these regions may alter the transcriptional response to exercise, mediating variation in performance and adaptation to training.

Circulating microRNAs expression in fit endurance horses during 90 km competition

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This study examined the full scope of ci-miRNAs through massive parallel sequencing in response to exercise in serum samples obtained from four competitive Arabian horses before and after the end of a 90 km endurance competition. The purpose was to identify putative biomarkers (ci-miRNAs) for predicting disease risks related to prolonged activity and monitoring metabolic adaptations. Blood samples were obtained by jugular venepuncture before exercise (T_0) and 2 hours after the end of the competition (T_1). All horses successfully passed the final veterinary examination. Ci-miRNAs were analysed at T_0 and T_1: NGS libraries were built from plasma derived RNA and sequenced producing 50 nucleotide Single-End reads that were cleaned and aligned to the reference genome. Changes in average haematological and biochemical parameters (Hct, Hb, RBC, WBC, CK, LDH. total protein) measured in blood samples reflects the physical and metabolic effort that occurred during the endurance competition. Differential gene expression analysis, Protein-Protein interaction network and significant enriched pathways of target genes were explored, and cluster of related targets with gene ontology enrichment was created. Our results show modulation of more than 100 miRNAs arising from tissues involved in exercise responses, such as muscle, heart, liver, and blood, and the modulation of correlated processes, including muscle remodelling and immune and inflammatory responses reflecting the acute response to endurance exercise in fit horses as consequence of cellular activation determined by prolonged aerobic exercise. For this, modulated ci-miRNAs in endurance athletes can be considered promising and reliable biomarkers of adaptation to prolonged physical activity.

Identification of candidate genes for hoof strength in Mongolian Horses

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Chinese Mongolian horses occupy diverse habitats, are less managed than modern Western breeds and are among the oldest surviving horse lineages. Long-term selection during the last 6,000 years for adaptation to local environments, including the ability to survive in extreme temperatures (i.e. -40 °C) and physical adaptations to the hoof for traversing rugged terrain, has led to several distinct Mongolian sub-types. We tested the hypothesis that genes contributing to hoof physiology may be identified in signatures of selection in the genomes of Mongolian 'Iron Hoof' horses that have a long history of selection by local herdsmen for tough hooves by comparing the genomes of 'Iron Hoof' horses (n=19) with other populations (n=81). Horses (n=156) from five distinct Chinese Mongolian populations (Abaga Black, Wushen, Wuzhumuqin, Sanhe and Baicha 'Iron Hoof') were genotyped with the Axiom MNEC670 Genotyping Array. After genotyping QC and removal of horses with close genetic relationships, n=100 horses were used. The Composite Selection Signals (CSS) method captured highly differentiated loci and variants with excess haplotype homozygosity in the target population ('Iron Hoof'). Using Ensembl Biomart genes were identified within ±1 Mb of selection peaks and DAVID (v6.8) enabled functional interpretation of the candidate genes. The CSS approach was validated by localising a genomic region on ECA22 harbouring the black coat colour gene (*ASIP*) in the Abaga Black population. For hoof adaptation, genomic regions harbouring genes (*RELA, B4GAT1, EXPH5* and *IL20*) with biological functions involved in keratinization were identified. These data will provide valuable insights into the functional mechanisms of hoof physiology.

Effects of inbreeding on sidebones and performance in a population of Cold-blooded trotters

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Ossification of ungular cartilages (OUC) is a common condition in several breeds. Recent studies state that the clinical significance of OUC have been over exaggerated and the heritability obscure. This makes elimination of stallions with OUC inappropriate. In fact, a growing body of evidence suggests OUC is a physiological adaptation developing early in life. The Cold-blooded trotter is a hard-working breed of sport horses with growing inbreeding as a potential risk for more diseases, and behaviour- and reproduction problems. Two parallel Swedish projects evaluated possible effects of inbreeding on performance and heritability of OUC in a population perspective. Different radiological findings of OUC and markers of performance for the entire careers of 645 horses, covering a 30-year period, were evaluated. Index of inbreeding was calculated from official horse data and statistics was performed using SAS, REG Procedure, and Model 2 ($P<0.05$). Calculations were performed both as a linear and as class divided variable (0-5, 5-10 and >10%). Inbreeding varied in the studied population between 0 to 24% (average 5.3%). There were no trends or significant correlations between inbreeding and different degrees of OUC or performance. In conclusion, the Cold-blooded trotter is numerically a relatively small breed with potential risk of several problems related to inbreeding. Our population is in many ways representative. Lack of correlations between OUC and inbreeding further strengthens the hypothesis that the development of sidebones is a physiological phenomenon in certain breeds of horses.

Impact of transport by road and air on heart rate in Icelandic horses

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Horses are transported by road and air all over the world for different purposes such as competitions. Approximately 1,500 horses are exported from Iceland every year by airplane. The aim of this study was to measure heart rate (HR) of horses in transport from Reykjavík (RVK) Iceland to Liege airport in Belgium. During the transport the horses travelled by road (Cherokee Warrior trailer) from RVK to Keflavík (KEF) and by airplane (Boeing 757 200 series) to Liege. Six horses (8±3 years) were evaluated and the transport was divided into 18 events. All six horses were equipped with HR meters (Polar Equine RS800CX). The transport from RVK to KEF took 45 min and the flight took 220 minutes. When the horses were not actively being moved they were waiting in the trailer or in special flight containers. ANOVA was performed including horse and event ($P<0.05$). Average HR during transport in horse trailer and airplane was not different (53±18 vs 64±22 bpm). The highest average HR was measured during airplane take-off (100±12 bpm) and landing (91±22 bpm). Loading and offloading on the trailer and container were the second most influential events of the trip (HR range 73-79 bpm). Interestingly HR was not different during the whole flight compared to resting HR in the stable before starting the transport (48±15 vs 33±8 bpm). It was concluded that during transport, HR of horses increases during airplane take-off and landing indicating some level of stress and/or increased muscle tension during these events.

Impact of using ear plugs during transport of horses

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Horses are transported daily on trailers all over the world for different purposes and very often to participate in training sessions and competitions. During transport, horses are subject to noise and other environmental factors that may cause stress. The aim of this study was to investigate the impact of using ear plugs for horses during short road transport. Eight riding horses aged 15±3.5 years were used in a crossover study. The horses were transported individually 25.4 km (29±1 min) in a trailer (Ifor Williams TA510G), once with earplugs (EquiFit T-foam ear plugs) and once without. The horses were fitted with Polar Equine RS800CX heart rate meters. For measuring noise, a Casella CEL-450 sound level meter was placed in the trailer. Data were analysed using ANOVA (proc GLM) and a model including horse, day and treatment. Average noise during the transport was 99 dB ranging from 51 to 121 dB. The result showed that fitting the horses with ear plugs lowered the average heart rate during transport by 10 bpm, from 70±15 bpm to 60±13 bpm ($P<0.05$). In conclusion, fitting horses with earplugs lowers heart rate during transport and is likely to reduce stress. This might have effects on sweat losses, competition focus and thereby performance in sport horses.

Gastric ulcers in eventing horses is affected by exercise intensity and housing

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Limited reports exist on gastric ulcer (EGUS) prevalence in horses competing in eventing. An online survey was administered to owners and trainers of horses competing in eventing to determine perceived prevalence of EGUS and to investigate whether competition level, training, nutrition and housing affected EGUS prevalence. The survey was administered to members of the United States Eventing Association via social media to individuals 18 and older. The study was approved by the Internal Review Board at all participating universities. A Random Forest model was used to identify influential factors associated with EGUS diagnosis and the most influential factors were assessed by combined logistic regression. Odds ratios were reported if $P<0.05$. Of the 1,336 horses represented, 44% were suspected of having EGUS. However, a veterinarian diagnosed only 22.3%, with 50.3% diagnosed by gastroscopy and 42.9% presumptive. The odds of having EGUS were higher for Thoroughbreds and increased by 4.0% with each year of age. EGUS odds also increased by 18% with each increased competition level and 0.8% with each hour housed in a stall. Odds of having EGUS increased by 0.3% with each percent of canter, gallop or jump added, whereas odds decreased by 0.1% with each percent of trot added when training. The apparent prevalence and odds reported here are a preliminary indication of links between exercise intensity and EGUS; however, they are likely most valuable in indicating the need for a prospective study to determine the true prevalence and risk factors of EGUS in eventing horses.

Biomechanics and Locomotion

The effects of head-neck position on the muscle activity of the long back muscle

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The goal of this study was to investigate the effects of two different head-neck-positions on the activity of the longissimus dorsi muscle. Fifteen warmblood horses were analysed walking and trotting on a treadmill. The data were collected at a natural (free and unrestrained) head-neck-position (HNP1) and then with side reins to place the horses' noses slightly in front of the vertical (HNP2). The horses were analysed in a fixed order (1. walk and 2. trot without side reins, 3. walk and 4. trot with side reins). Twenty surface EMG sensors (Delsys) were placed on the skin above the left and right longissimus dorsi from T12 to L3. The EMG system was synchronised with a kinematic camera system (10 cameras, Motion Analysis Corp.). Reference markers on the hooves were used to separate the EMG-data in motion cycles. Data processing was performed with MATLAB. For statistical analyses, ANOVA and a Wilcoxon test (SPSS) were used. No significant differences were found between the left and the right longissimus dorsi muscles. In walk, the maximum activity at T17 was significantly reduced (44%) by the use of the side reins (HNP2). In trot, the reduction caused by the HNP2 was more pronounced. We found significant differences at T12, T14, T15, T17, T18, L1 and L2, whereas the maximum effect was seen at T15 (reduction of 46%). This reduced activity of the longissimus dorsi muscle using HNP2 should be taken into account when training horses.

Three impulse-momentum models of the equine rotational fall

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The objective was to mathematically model the forces involved when horses galloping cross country strike an immovable fence. Three methods were used to model the horse-obstacle collision: single point mass estimation (PM); single rigid body estimation (FL); and multiple rigid body estimation (MS). Values were obtained from published research using motion capture to measure kinematics of the horse's centre of gravity, forelimbs, and hindlimbs at jumping take-off. A convenience method from video evidence of rotational falls was used to estimate time of collision (Δt) and rotational velocity of the horse about the collision point. Mean collision time (Δt=0.79 s) in PM produced horizontal, vertical, and resultant impact forces of 8,580 N, 8,245 N, and 12,158 N, respectively. Impact forces in FL were greatest at the proximal brachium (193.4 N<F_{avg}<1,362.4 N) and decreased along mid-brachium (189.5 N<F_{avg}<1,335.2 N), distal brachium (186.1 N<F_{avg}<1,311.5 N), mid antebrachium (179.6 N<F_{avg}<1,269.0 N), and distal antebrachium (173.5 N<F_{avg}<1,231.9 N). The greatest impact forces in MS occurred when colliding at the proximal brachium (5,739 N<F_{avg}<43,144 N) and decreased at the mid brachium (5,678 N<F_{avg}<42,385 N), distal brachium (5,626 N<F_{avg}<41,745 N), mid antebrachium (5,704 N<F_{avg}<35,136 N), and distal antebrachium (5,833 N<F_{avg}<34,871 N). Field validation in actual rotational falls is essentially impossible (impossible to predict and proactively instrument the horse before it falls). Computerized collision model validation is in progress but is incomplete at this time. For all models, shorter impact duration led to higher magnitude of force transfer between horse and obstacle. Transient impacts may tend to produce large instantaneous forces while slower, relatively more time-consuming rotational impacts may actually produce smaller magnitudes of force.

Towards standardised use of equine surface EMG: effect of normalisation and filtering on outcome measures

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Low-frequency noise attenuation and normalisation are fundamental signal processing (SP) methods for surface electromyography (sEMG), but are absent, or not consistently applied, to equine sEMG and their influences on outcome measures are unknown. During canter, leading hindlimb (LdH) experiences greater vertical loading than trailing hindlimb (TrH), thus differences in muscle activity are hypothesised. The purpose of this study was to determine the effect of normalisation and band-pass filtering on sEMG outcome measures, calculated from LdH and TrH data. sEMG (2,088 Hz) and 3D-kinematic (232 Hz) data were synchronously collected from right Biceps Femoris in 10 horses (9.7±2.6 years, 161.9±6.3 cm) during both canter leads (4.6±0.4 m/s). Two SP methods were applied to raw sEMG data; method 1 (M1) represents the most common SP method from equine literature (DC-offset removal, full-wave rectification). Method 2 (M2) includes additional high-pass filtering (Butterworth 4th order, 40 Hz cut-off), for artefact attenuation, and normalisation relative to maximum sEMG across all strides, to reduce inter-subject variability. Integrated EMG (iEMG), mean amplitude (MA) and peak amplitude (PA) were calculated using processed sEMG from both methods and stride duration as temporal domain. Data from LdH and TrH were grouped and compared within each method using repeated measures ANOVA. For M2, LdH was significantly greater than TrH for iEMG, MA and PA (P<0.01), whereas M1 showed no differences (P>0.05). Standardised equine SP is therefore recommended to detect differences that would otherwise have been missed. Further research is required to determine whether differences in muscle activity are due to differences in limb loading.

Collisional mechanics in the diagonal gaits of horses

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Collisional mechanics, which studies the redirection of the motion of the centre of mass by ground reaction forces (GRF) generated by the limbs during stance, is an important source of energy losses in locomotion. The objective was to compare collisional mechanics and the associated energy losses in horses performing diagonally-coordinated gaits over a range of speeds. Synchronized kinematic and GRF data were collected from four dressage horses performing piaffe, passage and trot at a range of speeds. Net collisional energy losses were determined from the sagittal-plane difference in the trunk velocity angle and the orthogonally-offset GRF angle by summation of mean losses during the absorption and generation phases. Net collisional losses were plotted against speed from 144 steps of data with speed range 0.001-5.12 m/s in a non-linear regression model. The best fit for the data was a logarithmic regression curve of $y = -0.384\ln(x) + 0.949$ (R^2=0.832, P<0.001). Net collision angles were piaffe 1.67±0.68, passage 0.79±0.12 and trot 0.55±0.06 (radians). Horses normally choose to make a transition to walk around 2 m/s and, as horizontal velocity decreased below this value in passage and piaffe, vertical velocity predominated and the trunk velocity vector became more closely aligned with the GRF velocity vector. As a result, net collisional energy losses increased greatly as horizontal velocity approached zero in piaffe. These findings illustrate that artificial gaits may lie outside the energy cost models for natural gaits and, as a result of collisional energy losses, may require greater energy expenditure than expected based on speed of progression.

Techniques for determining hoof movement during ground impact, using inertial measurement units

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Track surface conditions in Thoroughbred racing are known to have a significant impact on racing performance and the incidence of musculoskeletal injuries. Inertial Measurement Units (IMU's) containing three rate gyros and three accelerometers were attached to the dorsal hoof wall of the left front hoof with farrier's adhesive. Horses were galloped on the racetrack and data collected from the IMU's were used to measure hoof motion at impact to determine track conditions. Racetrack surfaces were rated independently by the track managers. Results for the mean hoof penetration for a galloping Thoroughbred over the last 800 m showed that two grass tracks with a rating of 'Good 3' were associated with average hoof penetration of 45 mm (SD 2.4) and 57 mm (SD 3.1) respectively, while the second grass track when it was rated 'Good 4' had a hoof penetration of 56 mm (SD2.3). A synthetic track had a mean hoof penetration of 83 mm (SD 4.3). Results gathered during this 'Track Rating System' project showed that the interaction between horse hoof variables (speed, acceleration and deceleration) and track variables (surface, hydration, consistency, compaction, cornering and camber), can be directly, quickly (within a few minutes), and conveniently measured using this non-invasive method. In contrast to previously published approaches, the loading completely represents the total limb loads in both magnitude and direction. Implementation of the Track Surface Rating System will also enable data to be collected to understand the effect of track surface conditions and surface type on musculoskeletal injuries.

Local unloading of subchondral bone results in a focal increase in remodelling in horses in training

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Bone remodelling is inhibited in high load environments such as in the skeleton of racehorses in training. Although it is assumed that this inhibition is a result of increased biomechanical strain within the bone, direct evidence for this mechanism is lacking. We aimed to investigate whether local joint surface unloading could offset remodelling inhibition in subchondral bone (SCB). An osteochondral defect was created arthroscopically in one surface of the midcarpal joint of six horses, which then underwent an eight-week training program. Osteochondral samples were collected from the site directly opposing the defect, from an adjacent loaded site and control samples from the sham-operated contralateral limb. Samples were imaged with microCT and backscattered scanning electron microscopy and sections stained with Masson's trichrome and TRAP. Results from treated and control limbs were compared using a paired t-test if differences were normally distributed and a Wilcoxon signed-rank test if not. In the SCB from the unloaded region there was evidence of increased remodelling reflected by increased active surface (mean difference=1.28 mm^{-1}, P=0.03), osteoclast numbers (mean difference=7.4 cells/mm^2, P=0.03) and osteoid width (mean difference=3.64 μm, P=0.04) with an overall decrease in bone volume fraction (median difference=-3.1%, P=0.04). In the adjacent SCB there was no change or the opposite effect (active perimeter: -0.66 mm^{-1}, P=0.01). Local unloading of the articular surface resulted in increased focal SCB remodelling in horses in training, evidence that increased cyclic strain on SCB has a direct inhibitory effect on remodelling and that SCB remodelling responds locally to changes in loading.

Objective detection and quantification of compensatory (false) lameness in horses

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The aim of this study was to assess prevalence and severity (LS) of compensatory lameness (CL) in horses evaluated with an inertial sensor-based system (ISBS). Data from 187 episodes of natural primary lameness (PL) (two groups: forelimb PL, 120 horses; hindlimb PL, 67 horses) evaluated with an ISBS before and after effective diagnostic analgesia of one limb. Changes in severity of forelimb ($LS_{forelimb}$ = Sqrt($MINDIFF_{head}^2$ + $MAXDIFF_{head}^2$)/6 mm) and hindlimb lameness (with hindlimb PL, $LS_{hindlimb}$ = |MINDIFFpelvis + MAXDIFFpelvis|/3 mm; with forelimb PL, $LS_{hindlimb}$=|$MINDIFF_{pelvis}$ – MAXDIFFpelvis|/3 mm) produced by diagnostic analgesia (DLS = $LS_{before\ analgesia}$ – $LS_{after\ analgesia}$) were used to investigate prevalence and severity of PL (DLS_{PL}>1) and CL (DLS_{CL}>0.5). Within each group, correlation between severity of CL and severity of PL was investigated with the Spearman's coefficient. Prevalence of CL was greater in horses with forelimb PL (60%) than in horses with hindlimb PL (32%). CL was usually less severe than PL. Severity of CL was unaffected by limb with PL, but was positively correlated with severity of PL. Hindlimb pushoff lameness had higher prevalence and severity than hindlimb impact lameness in horses with forelimb PL but not in horses with hindlimb PL. Knowledge that CL is usually less severe than PL and that, when there is forelimb PL, contralateral hindlimb pushoff CL is more prevalent and more severe than ipsilateral hindlimb impact CL may help clinicians distinguish PL from CL.

Sagittal plane fore hoof unevenness is associated with asymmetrical hindlimb frontal plane forces

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Asymmetry in forelimb dorsal hoof wall angles (DHWA), termed unevenness, is associated with lateralized grazing behaviour and sagittal forelimb gait asymmetries, but the effects on medio-lateral ground reaction force (GRF) production and hindlimb locomotion are unknown. This study investigated the effects of fore hoof unevenness on contralateral fore- and hind-limb force vectors patterns, in both sagittal and frontal planes. To retain left and right diagonal pair information, n=27 uneven fore-footed riding horses were classified into two groups: higher left DHWA (HIGH-LF; n=12) and higher right DHWA (HIGH-RF; n=15). Three-dimensional GRFs were collected during trotting. GRF summary vectors representing the magnitude (VecMag) and angular direction (VecAng) of the entire stance phase GRFs in both the sagittal as well as the frontal plane were calculated for each limb. Contralateral limb pairs were compared using ANOVA, separately for each of the HIGH groups (P<0.05). For the HIGH-LF group, the LF limb produced a significantly greater propulsive VecAng (Left; 1.44±1.00, Right; 0.64±0.80 (deg), P<0.05), whereas for the HIGH-RF group the RF limb produced a significantly greater medial VecAng (Right; 0.97±1.64, Left; -0.64±1.19 (deg), P<0.05). In both groups, a significantly greater lateral VecAng was found in the left hind limb compared to the right hindlimb. Production of notable medio-lateral GRFs in uneven horses during straight line trotting, particularly in the left hindlimb, suggests they have greater locomotory challenges beyond those previously described in the sagittal plane. These compensatory loading patterns may contribute to reduced longevity of uneven footed sport horses.

Prevalence of movement asymmetry in young Standardbred trotters

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Objective measurement of movement asymmetry for lameness evaluation is increasing. However, knowledge of biological variation of asymmetry in specific horse populations is lacking. This study aimed to describe the prevalence of asymmetry in young, untrained and presumed sound Standardbred trotters. Standardbred yearlings (n=87) from 13 trainers were included. These were measured during straight-line trot utilizing inertial measurement unit (IMU) technology. Four sensors mounted on the horse's head, withers, pelvis and right front leg, conveyed the degree of vertical displacement of the horse's body used to calculate asymmetry of the head (HD_{min}/HD_{max}) and pelvis (PD_{min}/PD_{max}). The resulting asymmetry scores (in mm) for the front- and hind limbs were evaluated based on previously established asymmetry thresholds: >6 mm for head variables, >3 mm for pelvic variables. Thresholds were surpassed for 79 (91%) horses for one or more parameters: HD_{min} (n=44, mean 14.1 mm, SD 7.3 mm), HD_{max} (n=35, mean 10.8 mm, SD 4.0 mm), PD_{min} (n=42, mean 5.9 mm, SD 2.1 mm), PD_{max} (n=48, mean 5.1 mm, SD 1.9 mm). Of these, 41 (52%) consistently had SD > parameter mean. Coexisting asymmetry of front-and hindlimbs was present in 45 (57%) horses. Almost all horses were above the applied thresholds, but variability in the measurements due to unsteady gait/head carriage was common. Knowledge of how asymmetry evolves with time and increased training may reveal its influence on performance and soundness. Information will be gained by following these horses over time.

Vertical movement symmetry and rider perceived laterality in elite riding horses

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Several populations of sport horses whose riders perceive them as non-lame have, however, been found to show relevant movement asymmetries. It is unknown if these asymmetries are related to orthopaedic pain or could be signs of motor laterality. The aim of this study was to compare vertical movement asymmetries across high performing elite sport horses and to explore the association of such asymmetries to rider-perceived laterality of the horses. Vertical movement asymmetries of head and pelvis were measured using IMU-sensors in 107 elite horses trotted in hand in a straight line. Trial means for differences in minimum and maximum positions of head and pelvis for the two contralateral steps per stride were calculated. The riders of 62 of these horses graded their horse's laterality during riding as none, mild, moderate or severe. In total, 77% of the elite horses showed one or several asymmetry parameters above the thresholds used for clinical lameness detection. Minimum position asymmetry of the pelvis was the most common asymmetry in the population (42%). This was the only parameter that differed between disciplines, with eventing horses being less asymmetrical (mean 1.6 mm) compared to dressage horses (3.4 mm) and show jumpers (3.1 mm) (Wilcoxon rank-sum, P=0.02). The association between the rider perceived laterality and objectively measured summed asymmetry parameters per horse (with values for head divided by 2) was not significant (Kruskal-Wallis, P=0.51). We conclude that vertical movement asymmetries above clinically used thresholds are common in elite horses and seem unrelated to rider perceived laterality.

The effect of saddle width on thoracolumbar range of motion

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To evaluate the effect of a too narrow or wide fitted saddle on thoracolumbar range of motion (ROM), in a crossover and interventional study, 13 non-lame horses (mean±SD: age 12±8.8 years; height 1.65±0.94 m) were ridden by the same rider. Tree width was confirmed as correct by five qualified saddle fitters and altered using an adjustable saddle (Kent and Masters). Horses were equipped with eight inertial sensors (poll, withers, thoracic thirteen [T13], thoracic eighteen [T18], lumbar three [L3], sacrum and left and right tuber coxae). Data were collected in rising trot with three repeats (sixty repeated strides) on both the left and right rein. Data were pooled (left and right) comparing tree widths (correct, narrow, wide) and analysed using a general linear model with repeated measures and Bonferroni post hoc (P<0.05). Compared to a correctly fitted saddle the following parameters were found; a decrease in ROM of T13 in a vertical direction for the narrow (P=0.003) and wide (P<0.0001) saddle; an increase in ROM of T13 in a latero-lateral direction was found for the narrow (P=0.004) saddle and decrease in the wide (P=0.001) saddle; an increase in latero-lateral ROM of L3 when fitted with a narrow and wide (P=0.02) saddle. Saddle width affects back kinematics and is therefore likely to alter locomotion. Saddles which are fitted too wide and/or narrow have an effect on thoracolumbar kinematics; these findings warrant further investigation along with the effect that saddle width has on the locomotor system.

Challenges of gait evaluation in endurance competitions according to FEI veterinarians

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The aims of this study were to assess the opinion of FEI endurance veterinarians (EV) about the challenges during gait evaluation and the occurrence of confrontations with riders when horses are eliminated from the races due to gait abnormalities and to assess EVs' feelings about the adoption of a user-friendly tool for objective gait evaluation to help EVs detect, quantify and document gait abnormalities. All FEI EVs were asked to complete an electronic questionnaire. There were 157 responses (25.8% and 40.1% of the EVs listed in 2013 and 2017, respectively). Most of the respondents were from Europe (56.1%) or the Middle East (16.6%). For the majority of respondents, detection of gait abnormalities is challenging, even for experienced and well-trained veterinarians (57.3%), and, many times, it is hard for the EVs to classify horses as lame or sound (65.8%). Handler not trotting the horse well during gait evaluation was considered the most common problem (94.3%) compromising the evaluation. Most EVs (98.2%) responded that they had been confronted at least once by the rider or associate about the decision to eliminate a horse simply due to gait abnormalities. Most EVs (71.3%) would be interested in having the support of a user-friendly tool for objective gait evaluation (33.3% for all evaluations; 38.0% only when horses have more subtle gait abnormalities). The findings of this survey suggest that it would be beneficial to use a tool to objectively detect and quantify gait abnormalities during endurance competitions to support the decisions made by the EVs.

The use of an artificial neural network to classify gait in Icelandic horses

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The Icelandic horse is a versatile horse remarkable for its five gaits of walk, trot, pace, tölt and canter. Gait classification is commonly performed visually, but this can be challenging. We hypothesised that an artificial neural network (ANN) could successfully classify all gaits. A group of 26 Icelandic horses were equipped with IMU sensors (sampling frequency 500 Hz, EquiMoves®). These sensors were attached to each metacarpal/metatarsal bone and one sensor to each hoof. A high-speed video camera was synchronised with the IMU sensors. Reference classification of each gait was performed from the video data by an Icelandic gait specialist. The classification was performed from steady-state gait using an ANN with one hidden layer of 45 neurons and with an input of 23 features. A total of 3,442 strides (walk=715, trot=516, tölt=1,218, left canter=381, right canter=396 and pace=216) were collected. Nineteen horses were used for training and the remaining horses for cross-validation. Overall, the ANN classified the gait correctly in 99.2% of the strides, only 0.8% were misclassified compared with the reference. The worst performance was in classifying pace and tölt, with 3 and 1.3% misclassification respectively. These findings demonstrate the excellent performance of objective gait analysis combined with an ANN for the classification of 5-gaited Icelandic horses. The technique will be very useful when evaluating performance, phenotyping of gait characteristics for genetic studies, or as a first step to develop algorithms for objective lameness assessment in the Icelandic horse for which correct classification of the gait is essential.

Comparison of visual lameness scores to gait asymmetry in racing Thoroughbreds during trot in-hand

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Measuring movement asymmetry with inertial measurement units (IMUs) in a clinical setting is practically possible. Normal ranges for specific cohorts of horses may be required to align expert veterinary assessment with IMU measurements for asymmetry screening. The aim of this study was to determine movement asymmetry values for lameness screening of Thoroughbreds in training compared to expert lameness scores. IMU gait assessment during in-hand trot-ups was performed in twenty-five racehorses undergoing routine gait analysis at the Singapore Turf Club. Six racehorse veterinarians graded video recordings numerically for lameness (0-5). Inter-observer agreement and consistency were determined. Sensitivity and specificity for head, withers and pelvic movement asymmetry were calculated from median lameness scores. Guideline values for aligning movement asymmetry values with expert opinions for both forelimb and hindlimb lameness were determined from sensitivity and specificity from receiver operating characteristics (ROC). Inter-observer agreement was poor to fair, and inter-observer consistency was good (intraclass correlation coefficient: 0.667 for forelimbs and 0.617 for hindlimbs). ROCs indicated higher discriminative power for hindlimb lameness using pelvic asymmetry (90% sensitivity, 93% specificity) compared to forelimb lameness using head asymmetry (69% sensitivity, 89% specificity) or withers asymmetry (44% sensitivity, 89% specificity). Compared to expert lameness scores from videos of a limited number of Thoroughbred racehorses, preliminary guideline values for movement asymmetry screening for forelimb lameness (>|14.5 mm|) and hindlimb lameness (>|7.5 mm|) are higher than previously reported clinical thresholds of >|7 mm| for head movement and >|4 mm| for pelvic movement asymmetry.

Objective pain assessment during rest and locomotion in horses with two types of induced lameness

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Study objectives were to assess the validity of the composite pain scale (CPS) and the facial assessment of pain (FAP) scale in horses with induced orthopaedic pain, as well as to investigate the performance of the FAP scale during walk and trot. Lameness was induced by application of sole pressure and intra-articular lipopolysaccharide (LPS) administration (2.5 ng) in eight Warmblood mares, including a two-week washout period between the two induction models. The horses were evaluated before and after lameness induction with inertial sensor (EquiMoves) and optical motion capture (Qualisys AB) systems. Direct pain assessment was performed in the stables by two independent non-blinded observers using the CPS and the FAP scale, and from the side during straight-line walk and trot on the hard and soft surface using the latter scale. Both scales showed excellent inter-observer reliability with a Cronbach's α of 0.99 for CPS and 0.93 for FAP (P<0.001). Differences in CPS scores were seen between baseline and 3-8.5 hours after LPS administration, and in FAP scores between baseline and 3-5 hours after LPS administration (Wilcoxon signed rank test, P<0.05). An increase in FAP scores was present in both gaits and models when comparing baseline with induced lameness (mixed models, P<0.0001). Both scales proved very useful in assessing induced orthopaedic pain in horses. Also, relevant FAP parameters could significantly describe the presence of alterations in facial expression during locomotion in lame horses.

Speed dependence of limb and body kinematics of horses walking and trotting on a treadmill

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Gait analysis parameters are sensitive to speed alterations. This study described changes in movement in 24 sound Franches-Montagnes stallions subjected to incremental speed tests at walk and trot. The speed ranges were adjusted individually and ranged from 1.4-2.0 m/s at the walk and 3.3-6.5 m/s at the trot with increments of 0.1 and 0.5 m/s, respectively. Limb and body kinematics were measured with 3D optical motion capture. The functional relationship between speed and kinematic parameters was tested using polynomial regression analysis. With increasing speed, limb pro- and retraction angles increased linearly in fore- and hindlimbs, at walk and trot; only walking forelimb maximal protraction angle remained stable at both ends of the speed range, but increased linearly in the middle. Maximal protraction height increased linearly in fore- and hindlimbs, at walk and trot. Maximal speed of limb protraction increased at walk linearly in fore- and hindlimbs; at the trot, the increase in the forelimbs was exponential; in the hindlimbs speed plateaued at higher velocities. Maximal fetlock hyperextension increased linearly in forelimbs at walk and in fore- and hindlimbs at trot; in hindlimbs at walk, it remained constant and decreased at higher speeds. Pelvis roll, pitch and yaw increased linearly at walk; at trot, roll angle was maximal and pitch minimal in the middle speed range; yaw started to increase only at the upper-end speeds. R^2 values of the majority of limb-related parameters (n=14 of 20) were ≥0.58 (0.58-0.94; mean 0.76). Subject speed affects limb and body kinematics. This knowledge allows for correction of non-speed-matched measurements.

Effect of performance level on pelvic vertical movement symmetry in horses trotting on a circle

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Lunging is used to evaluate lameness in horses. However, the circular path enforces a natural pelvic vertical asymmetry in sound horses. Also, it is important to identify additional influencers of natural pelvic movement asymmetry. Subsequently, the purpose of this study was to investigate whether elite performing riding horses show a different degree of pelvic vertical movement asymmetry compared to young horses in early training during lunging. Eighteen young riding horses (2-4 years) and seventeen adult elite performing horses were included, with symmetrical movement on the straight, verified with a sensor-based movement analysis system. Straight-line thresholds were set to ±3 mm for the over strides mean of the difference in the two maximal (PD_{max}) and two minimal (PD_{min}) pelvic vertical positions per stride. The horses were measured in both directions in trot on a 10 m circle, at a speed comfortable for the horse. The total degree of pelvic movement asymmetry during lunging was calculated by summing the absolute values for PD_{min} and PD_{max} respectively from the two directions. A comparison of directions was not performed. A two-tailed Mann-Whitney U test showed significantly greater total degree of PD_{max} asymmetry for the young (mean 6.33/SD 2.49) compared to elite performing horses (mean 3.74/SD 1.32; P=0.001). No significant difference was identified for PD_{min} (P=0.803). This study indicates that a greater natural pelvic vertical movement asymmetry can be expected in young horses compared to adult elite performing horses when trotting on a circle and should therefore not be misinterpreted as a push-off lameness.

Forehand drop in walking dressage horses on treadmill

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Kinematic asymmetries are used extensively in lameness detection at trot but have received little attention at walk. The objective was to seek asymmetries in vertical excursions of the withers during walking in horses that were sound at trot. Seven high-level dressage horses clinically sound during trotting were evaluated as they walked unmounted and unrestrained on a force-measuring treadmill. Twelve infrared optical cameras tracked reflective markers placed on standard anatomical landmarks. Mixed models were used to study associations between contralateral mean-trial differences (5-8 trials per horse) in minimal height of the spine at the sixth thoracic vertebra at early left vs right forelimb stance (T6minDiff) and spatiotemporal and vertical ground reaction force (vGRF, 1st and 2nd peak) variables. One horse was quite symmetrical, 5/7 horses consistently had T6 lower in early left fore stance, and one horse had T6 lower in early right fore stance. Trial-mean asymmetries ranged between 0.3 and 23 mm. When T6 was relatively lowest, the retracted forelimb showed increased retraction distance (+1 mm predicted +0.17 mm T6minDiff) and decreased stance duration (+1 ms predicted -0.3 mm T6minDiff) compared with the contralateral forelimb. The haunches were displaced toward the retracted forelimb, and the hindlimb ipsilateral to the retracted forelimb showed greater protraction and retraction distance (both +1 mm predicted +0.2 mm T6minDiff) than the contralateral hindlimb. There were no differences in peak vGRFs, suggesting that the kinematic asymmetries reflect sidedness rather than lameness. Further studies of locomotor asymmetries at the walk are needed both in sound and lame horses.

Withers movement symmetry can differentiate forelimb lameness from compensatory lameness in horses

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The objective of this study was to investigate whether the previously described compensatory asymmetry patterns in horses with induced lameness were evident also in horses with naturally occurring lameness. Three hundred and ninety horses, presenting for lameness at two European university equine clinics, were equipped with reflective markers at pre-determined anatomical landmarks. The data were collected using an optical motion capture system (Qualisys AB, Sweden) with the horse trotting in a straight line on a hard surface. The difference between the two vertical displacement minima of the head (HD_{min}), pelvis (PD_{min}) and withers (WD_{min}) was calculated for each stride. Horses with unilateral forelimb lameness (n=63) and horses with unilateral hindlimb lameness (n=36) where diagnostic analgesia decreased the lameness were included in the statistical analysis. Mean HD_{min}, PD_{min} and WD_{min} were compared before and after diagnostic analgesia using a paired t-test. Asymmetry of head and withers, but not pelvic, movement significantly decreased in the forelimb lame horses ($P<0.01$). For the hindlimb lame horses, all three variables decreased ($P<0.001$). Horses with forelimb lameness presented with unidirectional head and withers movement asymmetry, whereas horses with hindlimb lameness showed head and withers asymmetry of opposite directions and the head asymmetry was ipsilateral to the hindlimb asymmetry. This study illustrates how compensatory patterns in horses with naturally occurring lameness are similar to those in horses with induced lameness. Therefore, the direction of movement symmetry of the withers can be used to discriminate true forelimb lameness from compensatory head movement asymmetry caused by primary hindlimb lameness.

Vertical movement of head and pelvis in the Icelandic horse at walk, trot, pace and tölt

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Quantitative lameness assessment, utilising upper body symmetry measurements, is becoming popular in equine practice but the current systems are only validated for trot. Lameness assessment in Icelandic horses is challenging due to high stride frequency and gait transitions, making it difficult to identify the timing of footfalls visually. The association between the vertical movements of head and pelvis relative to the loading of the limbs in horses performing other gaits than trot is poorly understood. This particular knowledge is a prerequisite to identify the lame limb in gaited horses when head and pelvis movement asymmetry is evaluated. Twenty-six Icelandic horses were equipped with 12 IMU sensors (sampling frequency 500 Hz, EquiMoves®) and measured during walk, trot, pace and tölt while ridden. Stance and swing phase for each limb and the lowest/highest vertical position of the head (H_{min}/H_{max}) and pelvis (P_{min}/P_{max}) were calculated. H_{min}/P_{min} events occurred at 55.0%/17.9% (walk), 46.5%/65.9% (trot), 44.4%/66.7% (pace) and 52.1%/60.3% (tölt) of the stance phase of the forelimb/hindlimb. All H_{max}/P_{max} events occurred within the last 10% of the stance phase, during the suspension phase, or during the first 10% of the stance phase of the next limb, except for P_{max} at walk (75.7% of stance phase). To conclude, the H_{min} and P_{min} were closely related to midstance of the fore and hindlimb respectively in all gaits, except for the P_{min} at walk. Therefore, changes in vertical movement symmetry for H_{min}/P_{min} are probably good indicators of weight-bearing lameness. P_{max} is probably a good indicator of push-off lameness, except at the walk.

Quality measures of correct diagonal stance detection in trot based on pelvic markers

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Quantitative lameness assessment using 3D optical motion capture is simplified if only upper body markers are used. The challenge with this approach is to correctly classify left/right stance. This study investigated a novel method to estimate the likelihood of correct left-right stance classification in horses at trot based on pelvic movement. This study used optical marker based locomotion analysis with three markers on the pelvis on a total of 9,351 measurements (>8 strides) in horses trotting on different surfaces, on straight lines and left/right circles. For approximately 7% of the measurements, there were also limb markers enabling automatic control of correct diagonal stance. A linear mixed model analysis was used on 18 selected pelvic locomotion characteristics to create a certainty value; a consistency value was also constructed, both normalised to 0-100%. The algorithms were applied to all measurements, and the 12% lowest consistency and certainty values were manually controlled for correct classification from video recordings. Based on the decrease of erroneous classification by increased consistency and certainty, correlation between consistency/certainty and risk of faulty classification were calculated. At consistency 78% and certainty 22%, the risk of faulty classification was less than 0.5%. Since limb movement defines gait, left-right step detection using only upper body markers is challenging unless limb movement is measured directly. Therefore, users of automated systems should always be responsible for controlling left/right classification. With a multi-trait based automated system, it is possible to reach very high accuracy (99.5%) in left/right detection using defined quality thresholds.

Variation in gait symmetry parameters in sound horses at trot on the straight line and on the lunge

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Knowledge of normal variation is mandatory for correct interpretation of quantitative gait data. This study investigated between-trial, between-day and between-horse variation in locomotion symmetry at trot. We hypothesised a significant amount of between measurement variation (BMV). Twelve competition-sound horses were trotted on a straight line and on the lunge, which was repeated 12 times over 3 days with 5 and 10 minutes in between. Data were collected overground, using optical-motion-capture (28 x Oqus-700+, 100 Hz) and RUD/RDD (difference in upward/downward movement between right-left halves of a stride), and MinDiff/MaxDiff (difference between left-right stride half-cycle in maximum/minimum vertical position) were calculated. For each horse, symmetry parameter and trial condition, the mean deviation (difference from each trial to the average of all trials) of all trials was calculated, resulting in an offset adjusted average measured asymmetry. BMV was analysed by calculating the prediction intervals from all horses for each condition and parameter separately. A linear mixed model was used to test for the effect of time, surface and path (straight line and circle). BMV: (MinDiff, MaxDiff, RUD, RDD), 14.4, 12.0, 19.9, 15.5 mm (head); 3.9, 3.0, 5.4, 4.2 mm (withers) and 4.2, 3.6, 2.2, 8.3 mm (pelvis). There was no significant effect of time (within and between days), surface or path. Between-horse variation was larger than within-horse variation. A substantial BMV can be expected from sound horses, especially for the head. During interpretation of clinical data, these results should be acknowledged especially when interpreting repeated measurements, as commonly done in routine lameness work-up.

A finite element model of an equine stifle joint

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The aim of this feasibility study was to develop a finite element model (FEM) of an equine stifle and simulate different load scenarios via finite element analysis. A series of sectional MRI (1.5 T) of the knee joint of a 23-year-old euthanatized Shetland pony gelding served as basis for the development of the FEM. The femur and tibia including their articular cartilage surfaces, the menisci, both collateral ligaments and the meniscal ligaments except for the meniscofemoral ligament were comprised in the FEM model. The DICOM series was processed using AMIRA software to create finite elements. The volume objects were adapted by FEBio software in order to get a functional FEM. FEBio was also used to run the simulation and analyse the results. Tissue material properties data were adopted from human tissues (Open knee project) except for the meniscal ligaments, for which bovine data were implemented. A functional FEM model of the equine stifle joint was developed with a range of motion of approximately 30°. Simulation results were evaluated by comparing the deformations to the data of a material testing machine. The model showed higher translocation and deformation throughout the range of motion in the lateral compared to the medial meniscus. As expected, the deformations were smaller (<5 mm) in comparison to the tested menisci of large horses. In this feasibility study a FEM prototype of an equine knee joint was successfully created. The results encourage further development of this FEM model to study the effect of shoeing and congenital malalignments.

Objective detection and quantification of irregular gait in endurance competition

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Detection of irregular gait (IG) during endurance rides is based on a brief subjective examination. Horse elimination due to IG is common and affects the outcome of the rides. The aim of this study was to investigate the feasibility of objectively detecting and quantifying IG with a portable inertial sensor-based system (PISBS) during an endurance ride. All 29 competitors of two simultaneously held qualifying endurance rides (40 km [stop at 20 km]; 80 km [stops at 40 km and 60 km]) were invited to participate in a study where gait was simultaneously assessed by FEI veterinarians and the PISBS. Asymmetric vertical displacement of the head and/or pelvis measured by the PISBS indicated IG severity and identified the affected limb(s). All veterinarians and competitors were asked to complete questionnaires about the use of the PISBS. The PISBS detected IG in 21/22 horses (48/70 evaluations [2-4 evaluations/horse, median=3]). Significant ($P<0.05$) disagreement between the PISBS and veterinarians was detected. Disagreement between the PISBS and veterinarians was no longer detected ($P\geq0.05$) after reducing the sensitivity of the PISBS by reclassifying horses with mild IG (IG<2× threshold) according to the PISBS as sound. Questionnaires were completed by 4/4 veterinarians and 17/22 competitors and all had favourable impressions about the use of the PISBS, but recommended reducing instrumentation time and trotting distance for expediency. Inherent human limitations may explain the lower sensitivity of veterinary evaluation relative to PISBS evaluation. It is feasible to use a PISBS for detection and quantification of IG during endurance rides. Simple methodological changes are likely to address the issues raised by competitors and veterinarians.

Assessing limb segmental accelerations during equine water treadmill exercise

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The aim was to evaluate the segmental accelerations of the equine forelimb under various water height/speed combinations in a water treadmill (WT). Five horses were used in a controlled study. Three uniaxial accelerometers (sampling rate: 2,500 Hz) were secured on the left forelimb (hoof, mid-cannon, and mid-radius). Horses walked at two speeds (S1:0.83 m/s, S2:1.39 m/s) and three water heights (mid-cannon, carpus, stifle), with a dry WT control. Peak acceleration of each segment was averaged over five strides. Stride frequency was calculated. Comparisons were determined using Friedman's tests ($P<0.05$). Comparisons between limb segments: Control: Peak acceleration was greater at the hoof than at the radius (S1:$P=0.005$, S2:$P=0.03$). Water: Accelerations for all water heights and speeds were significantly greater at the cannon compared to the radius ($0.005>P<0.05$), with no differences between the hoof and cannon, or hoof and radius. Comparisons within limb segments in all WT conditions: The dampening effect of water was only significant at the hoof (stifle water height) (S1: 2.76 G median decrease (1.96-5.63, $P=0.04$); S2: 2.11 G median decrease (1.92-2.35, $P=0.02$). A greater stride frequency was observed with water at the height of the carpus ($P=0.04$) and stifle ($P=0.02$) for S2. Effect of speed: For any given water height, speed had no effect on peak acceleration. In all conditions, hind hoof strike induced front segmental accelerations of the same order as forelimb strikes themselves. The dampening effect of water on forelimb varies such that the accelerations experienced by the cannon bone are the greatest. This should be considered when designing water-based equine rehabilitation protocols.

Converging-diverging shape configuration of the diaphysis of equine third metacarpal midshaft bone

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The shape of the diaphysis of the equine third metacarpal bone (MC3) substantially influences its mechanical properties. The connection between bone shape and bone growth is likely to be useful in forecasting the response of MC3 to a training program and predicting its internal loading. The areas in three cross-sections (real slices with the marrow area subtracted) of the diaphysis of 27 Thoroughbred horses with a wide range of ages (<6 months, n=4; 17-20 months, n=6; 2 years, n=6; 3 years, n=4; 4-8 years, n=7) were measured followed by creating the surfaces of the slices using computer-aided-design. Results were then obtained using a statistical MATLAB code. For all the samples, the values were plotted for a slice taken from the mid-point of the shaft, and for two others located 3 cm proximally and distally from the middle slice. The area of the slices decreased from proximal to distal in the majority of the specimens, yet in all foal samples the area fluctuated and showed a converging-diverging shape along the midshaft of MC3. A similar trend was observed for one adult sample. Previous studies have shown that 0.5 mm differences in thickness of the dorsal cortex have a significant effect on local strain *in vivo*, and have investigated the significance of shape variation in the diaphysis of MC3. Increased surface strain on the MC3 midshaft, observed in equine training, causes remodelling and changes in shape. Hence, comprehending the relationship between the bone's shape and loading is required to determine what sort of exercise is required to prepare the MC3 for racing.

The role of accurate stride analysis in understanding the contribution of limb loading to injury

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This work aims to provide trainers with ways of identifying and managing horses at increased risk of injury by integrating conformational data with limb loading data collected during training. Techniques for fatigue and failure management in mechanical structures rely on understanding the external loading environment, the internal load paths, features where high stresses concentrate, and the material characteristics. A method of measuring stride characteristics and hence determining limb geometry during gallop is described, which is an important first step in determining limb loading. Limb motion was measured at gallop on a racetrack using inertial measurement units, comprising three orthogonal accelerometers and three rate gyroscopes attached to the cannon of all four limbs. Using standard six-degree of freedom kinematic equations of a rigid body, which relate acceleration, velocity and displacement, the angular positions of the limb were reconstructed. From mid-stance limb angle, estimates of limb angles during constant speed gallop over 10 strides were derived for maximum protraction (48.0° SD 3.1) and retraction (55.0° SD 3.8), limb angle at hoof impact (31.5° SD 1.8)and hoof roll-over (34.2° SD 2.1). The model assumed body mass loads predominantly as compression through the limb bone structure and that, during stance, the load path can be represented by a single linear force vector between hoof and body. Data from this stride analysis will be used in a model of locomotion to determine limb loads during gallop. The knowledge of limb loading will be integrated with limb conformational data to explain limb injury and failure.

Induced rider asymmetry and its effects on equine limb kinematics and thoracolumbar range of motion

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To evaluate the effect of rider asymmetry on horse locomotion, 10 rider-sound horses (mean±SD: age 11±3 years; height 1.50±0.67 m) participated in an interventional study. Twenty-four joint centre markers were used to capture 2D kinematics (Quintic Biomechanics) with a high speed camera (spatial resolution 1,300×400, 400 fps at 10 m distance) recording left and right lateral views. Horses were equipped with eight inertial sensors (poll, withers, thoracic eighteen, lumbar three, tubera sacrale, left and right tuber coxae and caudal sacrum). After a rider prescribed warm up period lasting 15 minutes, data were collected in rising trot from three passes on both the left and right rein for both symmetrical and asymmetrical conditions. Rider asymmetry was induced by shortening the left stirrup by 5 cm. A paired t-test was used to determine differences between conditions ($P<0.05$). With shortening the left stirrup, inducing rider asymmetry, the following parameters showed a significant increase: laterolateral range of motion at the withers ($P=0.02$); third lumbar vertebra ($P=0.02$); left tuber coxae ($P=0.04$) and craniocaudally ($P=0.04$); right front fetlock hyperextension ($P=0.01$); left carpal flexion ($P=0.04$) and right hind fetlock hyperextension ($P=0.04$). These changes are likely to be gait adaptions in response to the asymmetric rider. In conclusion, asymmetric rider position has an effect on thoracolumbar range of motion and limb kinematics. Riders should consider the effect that their position/asymmetries have on the locomotor system. These findings warrant further investigation to understand the long-term impact this has on gait symmetry.

Subjective and objective evaluations of horses for a fit-to-compete or unfit-to-compete judgement

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Each horse at an FEI competition must pass a veterinary inspection for 'fit-to-compete' or 'unfit-to-compete' judgement. However, different FEI Veterinary Delegates (VDs) often have varying opinions. The objectives of this study were to evaluate the intra/inter-observer agreement of 'fit-to-compete' judgements and compare these with objective gait analysis measurements. Twelve horses were evaluated by three experienced VDs and one veterinary specialist (dipECVS) and video-recorded for re-evaluation later. Measurements with a quantitative-gait-analysis system (Qhorse®, Qualisys) were performed and left-to-right symmetry of head and pelvis was calculated as the vector sum of the difference between the two vertical displacement minima and maxima of the head (VS_HD) and pelvis (VS_PD). The Fleiss Kappa inter-observer agreement from the live-evaluations between the four veterinarians was fair ($\kappa=0.395$; $P<0.001$). Video-evaluation differed from live-evaluation on 11 occasions (for 3 vets 3 cases, for 1 vet 2 cases, in total 23%): 7 assessments changed from 'unfit-to-compete' to 'fit-to-compete' and 4 from 'fit-to-compete' to 'unfit-to compete'. The Cohens Kappa for intra-observer agreement between live observations and videos after one month was also fair ($\kappa=0.345$). Of all horses classified as 'fit-to-compete' by all judges, VS_HD and VS_PD (mean±SD) was 11.0±4.3 mm and 3.0±1.2 mm, respectively. Of all horses classified as 'unfit-to-compete' by at least one judge, VS_HD and VS_PD (mean±SD) was 18.8±12 and 5.6±4.7 mm, respectively. In conclusion, although horses unanimously scored 'fit-to-compete' had lower asymmetry scores in quantitative gait analysis, the 'fit-to-compete' or 'unfit-to-compete' judgement proved difficult even by experienced veterinarians.

Radiographic evaluation of equine tarsus morphometry

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The equine tarsus is the most common area of the hind limb associated with lameness. Tarsus conformation is probably directly related to its soundness and function. Previous conformational measurement methods and radiological data are either limited or too specific to explain the complex tarsus conformation in different loading conditions. This study aimed to develop a consistent method to evaluate equine tarsal conformation from lateromedial (ZLM) radiographs. Twenty cadaveric hind limbs from 13 adult horses of different breeds were cut at the distal one third of the tibia. Hind limbs mounted in a loading rig and positioned with the metatarsus vertical were digitally radiographed. The zero-degree lateromedial (ZLM) was defined by vertical and horizontal landmarks including overlapping of the lateral and medial trochlea of the talus and a contact point between the dorsal edges of lateral and medial borders of the distal central tarsal bone. Radiographs missing these features were retaken to achieve consistent ZLM views. Specific radiographic features were selected as landmarks for developing tarsal parameters based on clarity and being consistently identifiable. The intra-rater repeatability of ten measurable morphometric parameters was evaluated with each radiograph measured twice with an interval of at least one month and Bland-Altman plots developed from this data. Repeat measurements did not differ significantly (ICC ranged from 0.766-0.970). This study provides a base for evaluation of the tarsal conformation by radiography.

A dynamic computational model of whole-body equine locomotion

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A computational model of the whole body of the horse is presented, which can be used as a virtual experimentation tool for evaluating changes to track properties on the kinematics and internal loads for racehorses. Kinematics of three Thoroughbred horses (mass 488±12 kg) galloping on a treadmill were measured using 86 markers and 19 motion capture cameras (Vicon Motion Systems, UK). Joint angles and centre-of-mass translations were calculated using custom optimisation-driven inverse kinematics code and subject-specific skeletal models comprising 42 joints and 43 body segments. Body segment inertial properties were scaled from regression tables available in the literature. A visco-elastic model of the treadmill surface was developed and then coupled to the skeletal model using a nonlinear contact formulation. Dynamic simulations of each gait were developed using the subject-specific models. Centre-of-mass rotations and translations and joint angles were predicted for each time step. Model and experimental results were compared to evaluate accuracy. Distal net joint torques, muscle forces, and joint contact forces were calculated for each time step of the simulations and found to increase in magnitude with galloping speed. Use of the model to investigate changes to ground surface on joint loading was demonstrated using a range of stiffness and damping parameters to understand their effects on kinematics and internal forces. This is the first model to use a forward dynamics simulation of the entire body to predict ground reaction forces, deformation of the ground surface, and dynamics of the distal forelimb simultaneously and enables virtual experimentation with ground surface, conformation and kinematics.

Longitudinal objective monitoring of locomotor symmetry in trotting young Warmblood horses

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Temporal stride kinematics of young Warmbloods have been reported on a treadmill using wired systems. To date, wireless optical motion capture (OMC) systems are routinely used to objectively measure the symmetry of a horse's locomotion. Therefore, this study aimed to longitudinally monitor young Warmblood horses trotting in hand, and it was hypothesized their symmetry would not change over time. The trot of twelve 5-year-old high-class Warmblood horses was measured using an n=18 OMC system (Qhorse®) at three consecutive times, before and after three months of training, and after showing. Temporal stride and symmetry kinematics were analysed using a linear mixed model, while the ICC was used to assess the consistency of the measurements at the three different time points (95% confidence interval). Concerning temporal stride kinematics, stride frequency (1.4±0.1 Hz) and velocity (3.4±0.2 m/s) decreased significantly after training (1.3±0.07 Hz, 3.2±0.2 m/s, respectively; $P<0.05$) and increased after showing (1.4±0.1 Hz, 3.6±0.5 m/s, respectively; $P<0.05$). Stride length (2.8±0.2 m; 2.7±0.3 m, 2.9±0.3 m), as well as symmetry kinematics were not statistically different between the three-time points. The ICC of the difference between the maximal vertical upward movement of the head was 0.87 (0.63-0.96) and of the withers 0.91 (0.74-0.97). For downward movement the ICC of the difference between the vertical displacement minima of the pelvis was 0.93 (0.80-0.98). Symmetry parameters of these young sport horses did not change over time, while the ICCs of upward and downward movement symmetry variables were high after several months of training, similar to reported previously for daily repeats.

Validation of gait event detection algorithm using hoof-mounted inertial measurement units (IMU)

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Inertial measurement unit (IMU) sensors are versatile and affordable tools for gait analysis. The objective of this study was to validate a hoof-on/hoof-off detection algorithm for hoof-mounted IMU sensors. Tri-axial acceleration (accel) and rate-of-turn (RoT) were measured with IMU sensors (sampling frequency 200 Hz, Inertia-Technology) attached to the lateral quarter of the right front (F) and hind (H) hooves of seven Warmblood horses. As a gold standard, horses were walked and trotted over the force plate (sampling frequency 200 Hz). Axes were synchronised by calculation of the root of the sum of squares resulting in one-directional IMU signals. Algorithms to detect hoof-events based on peak detection were developed; a threshold of mean+2.58×SD was used for the vertical force and mean+1.96×SD for the IMU data. The accuracy and precision of these algorithms was calculated as the mean time of FP minus IMU in milliseconds (ms) and the SD of these differences. At total of 152 steps (36-walk and 40-trot for both F and H) were analysed. For hoof-on, accuracy in accel/RoT were 11/87 ms (F), -16/79 ms (H) and for precision 29/66 ms (F), 15/98 ms (H). For hoof-off, accuracy in accel/RoT were -91/-11 ms (F), -142/-15 ms (H) and for precision 108/14 ms (F), 140/23 ms (H). Hoof-on events were detected too early by both IMU algorithms and hoof-off events too late. These preliminary results show that combining these IMU algorithms is very promising for gait classification. Further algorithm development will include break-over phase detection to improve hoof-off accuracy and hoof-event detection on soft surfaces.

Kinematics of marcha picada of Mangalarga Marchador horses with AA and AC genotypes of DMRT3

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Considering the effect of the DMRT3 gene on the gait pattern of the Icelandic, Finnhorse and Standardbred horses, the present investigation was designed to evaluate the influence of the DMRT3 genotype on the kinematic variables of the marcha picada (MP) gait of Mangalarga Marchador (MM) horses. Eighteen horses were divided into two genotype groups: AA (n=9) and AC (n=9). Eighteen optoelectronic cameras (240 Hz) acquired three-dimensional kinematic data of markers fixed to the hooves of horses ridden over a 22 m track in marcha gait. Duration, length, frequency, and velocity of the strides, diagonal advanced placement and lift-off and support phases (diagonal, lateral, tripedal and monopedal) were calculated. Individual data were the means of each variable over four strides in the middle of the track. Data were transformed, and groups were compared by t-test (P<0.05). The AA group presented higher diagonal advanced placement at lift-off (P=0.037; AA=31.74±2.39%; AC=28.91±2.87%) and lower diagonal supports (P=0.045; AA=35.52±4.58%; AC=40.98±5.97%). This finding might be explained by the role of DMRT3 in left/right and fore/hind limbs coordination. However, differences were observed only in two kinematic variables, suggesting that additional genes also determine the movement pattern or interfere with the DMRT3 gene, apart from the influence of environmental factors. Nevertheless, the results suggest some relationship between different DMRT3 genotypes and the kinematic variables in the MP of ridden MM horses.

Objective evaluation of stride parameters in the five-gaited Icelandic horse

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Evaluation of gait quality in the Icelandic horse at breeding shows and in competitions has so far only been based on subjective judging scales. The aim of the study was to provide quantitative data for temporal stride parameters for the five gaits of the breed. Twenty-six Icelandic school horses, ridden by experienced riders, were equipped with inertial measurement unit (IMU) sensors (EquiMoves®) that were attached to each metacarpal/metatarsal bone and set to a sampling speed of 500 Hz. A video camera was also synchronised with the IMU sensors. Representative strides (>200) for each gait were selected from the videos by a qualified judge and stride parameters were calculated in descriptive statistics in Minitab based on hoof-on and hoof-off IMU data on a stride per stride basis. Mean±SE for each gait were: *Walk* (715 strides): duty factor front limbs (DF-front) 62.6±0.09%, duty factor hind limbs (DF-hind) 59.0±0.06%, lateral advanced placement (LAP) 25.6±0.12%. *Trot* (516 strides): DF-front 43.9±0.18%, DF-hind 45.8±0.23%, suspension 3.5±0.22%, diagonal advanced placement (DAP) 4.9±0.19%. *Tölt* (1,218 strides): DF-front 45.1±0.14%, DF-hind 44.8±0.12%, LAP 19.4±0.21%. *Pace* (216 strides): DF-front 35.7±0.28%, DF-hind 42.1±0.41%, suspension 9.9±0.56%, LAP 12.5±0.52%. *Canter* (777 strides): DF-front 39.3±0.14%, DF-hind 43.8±0.19%, suspension 7.1±0.22%. Valuable quantitative data for several important stride parameters of all five gaits of the Icelandic horse were collected in a field setting using IMU sensors. In combination with traditional subjective methods, this objective technique might enhance assessment of gait quality in competitions and breeding shows.

The effect of trotting speed on the kinematics of head, withers and pelvis

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Quantitative kinematic gait analysis is gaining popularity in equine practice. It allows for objective quantification of motion symmetry, which can be used to quantify the effect of nerve blocks and flexion tests. This approach is mainly based on measurement of motion symmetry of the vertical displacement of head, withers and pelvis. The objective of this study was to evaluate the effect of trotting speed on kinematic symmetry parameters in a group of horses perceived to be sound by their owner. Thirty-six horses were equipped with reflective spherical markers on the poll, withers and tuber sacrale. After an acclimatisation period to treadmill locomotion, horses were trotted on the treadmill at increasing speeds up to their individual breaking speed at increments of 0.4 m/s. Kinematic gait analysis was performed using 3D optical motion capture. The effect of speed on several kinematic parameters was tested using mixed models with significance set at $p < 0.05$. For the head, increased speed resulted in a significant reduction of the asymmetry parameter vector sum (VS) ($P<0.001$) and a decrease in the range of motion (ROM) of the vertical displacement ($P<0.001$). For the withers, the effect was a decrease of the withers VS ($P<0.001$) and an increase of ROM ($P<0.001$). For the pelvis, an increase of speed resulted in a reduced ROM ($P<0.001$), but no significant effect was found for the pelvis VS ($P=0.7$). Speed has a significant effect on important kinematic parameters used for objective lameness assessment. Speed between repeated measurements should, therefore, be maintained as constant.

Histological and molecular characterization of equine myofascial structures

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The importance of myofascial structures for locomotion and performance has become increasingly clear, but few studies are available on equine myofascia. The aim of this study was to provide a characterization of equine myofascia to improve understanding and assessment of myofascial function. Myofascia was collected from 10 areas (representing myofascia keypoints, joint-related areas, and neutral areas) in six horses and processed for histology. Three areas were also divided in superficial and deep regions and processed for mRNA. Histochemistry included haematoxylin/eosin, Resorcin fuchsin/van Gieson, Alcian Blue for hyaluronic acid, and immunohistochemistry for S100 (nerve fibres), and αSMA (muscle fibres, myofibroblasts). Quantitative PCR was performed for mRNA expression of smooth muscle (αSMA, Calponin 1), neurons (S100B, nNOS), mechanoreceptors (Fam38a and b), and collagen type 1 and 3 (COL1/3). Histological analysis showed clear regional differences in morphology with marked demarcations of layers, including superficial fascia, deep fascia, and interspersed layers of areolar tissue, adipose tissue, and epimysium. Differences were not related to initial selections, but rather to functional properties related to myofascial lines. S100 staining in areolar tissue followed a horizontal plane between superficial and deep fascia, a vertical plane along the retinaculae, and around blood vessels. αSMA staining was inconclusive. mRNA analysis showed large variation between samples. Expression of αSMA and Calponin 1 was higher in the superficial fascia. Mechanoreceptors, neurons, COL1, and COL3 were expressed, but with no clear pattern. In conclusion, these results provide a characterization of equine myofascia, supplemented with S100 staining pattern.

The dilemma of the overfed Shetland pony: subclinical laminitis vs optimal showing

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Traditionally, Shetland ponies are overfed for optimal studbook showing, putting them at increased risk for developing laminitis. This study compared the trot of normal vs obese Shetland ponies using objective gait analysis equipment in order to identify locomotor parameters that may be used as early indicators of subclinical foot pain. Ten adult Shetland mares (mean±SD: 5.0±2.1 years) were randomly (n=5) assigned to two different diets for 8 months resulting in a healthy (100% ratio: 189±15 kg), and an obese (200% ratio: 247±42 kg) group. An n=18 infrared (IR) camera optical motion capture system (QHorse®; 200 Hz) and n=3 reflective markers at the head, withers, and pelvis were used to objectively assess kinematics before and after a bilateral diagnostic nerve block of the forefeet ($P<0.05$) at similar speeds (before: 2.23 m/s, after: 2.24 m/s). In the obese group diverging hoof rings and dorsal hoof wall pain percussion scores were significantly higher ($P<0.05$). After blocking, stride duration (before: 0.51 s, after: 0.55 s) and range of downward and upward vertical movement at the withers significantly increased (before: 69.0 mm, after: 77.3 mm; $P<0.05$) in the obese group (normal group before: 0.52 s, after: 0.52 s; before: 83.1 mm, after: 76.4 mm, respectively, all NS). Apparently, traditional doubled feeding put the obese group at risk for painful feet, illustrated by their positive blocking response. It is concluded that these parameters are useful for monitoring subclinical foot pain. Further, when preparing for studbook showing, prevention of obesity seems a more optimal preparation than fattening these animals.

The effect of NSAID treatment on movement asymmetries in riding horses

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When quantitative gait analysis is performed in horses perceived as free from lameness by their owners, 73% present with movement asymmetries of equal magnitude to horses with low-grade lameness. Whether these movement asymmetries are related to orthopaedic pain and/or pathology is still unknown. Therefore, the objective of this study was to determine whether motion asymmetries in 'owner sound' horses are affected by anti-inflammatory treatment. Vertical movement asymmetry was measured in straight-line trot using uni-axial accelerometers. For all strides the mean difference between the two displacement minima and maxima of the head (HD_{min}, HD_{max}) and pelvis (PD_{min}, PD_{max}) per stride were calculated for each trial. Horses (n=65) with asymmetries above thresholds: HD_{min}/HD_{max} of >±6 mm or a PD_{min}/PD_{max} of >±3 mm, were included in an owner-blinded study and treated with meloxicam and placebo for four days each in a crossover design. Measurements on a hard and a soft surface at two speeds ('preferred' and 'fast') were carried out just before and on day four of each treatment with a 14-16 day washout period in between. A mixed model analysis was performed with the sum of the absolute values of $HD_{min}/_{max}$ and $PD_{min}/_{max}$ (with head variables divided by 2) as outcome, treatment, stride frequency and surface as fixed effects and horse as random effect. To conclude, treatment with meloxicam did not significantly decrease the movement asymmetry ($P=0.12$). These results raise new questions whether such asymmetries represent normal biological variation or are related to chronic pain/dysfunction not responsive to a four-day meloxicam treatment.

Asymmetries in hoof shape and differences in pressure distribution under the hoof

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Six Welsh-type horses were used to explore front hoof asymmetries and pressure distribution under the hooves. Hoof parameters assessed were proximal hoof circumference (PHC), hoof angle (measured with hoof gauge), solar perimeter and solar area (calculated from photographs). Pressure data were collected while each horse was led walking unshod over a Tekscan pressure mat (Tekscan Inc; South Boston, MA, USA; 100 Hz). The averages of five clean foot strikes for each hoof were used to obtain peak force and contact area. A paired t-test compared variables for the left and right hooves. Four horses had a right larger PHC, one a left larger, and one was equal. This difference (11.25±6.09 cm) was significant (P=0.035). Four horses had higher right hoof angles, two were equal; mean difference was 2.2±2.1° (P=0.052). Four of the horses had a larger solar area of their right hoof, and two had larger left, a difference of 7.93±4.1 cm^2 (P=0.005). Four horses had higher peak force under the left hoof, two under the right; a difference of 381±288 N (P=0.023). Four horses had a larger contact area under the left hoof, two under the right; a difference of 11.25±6.09 cm^2 (P=0.006). Five of the horses had a higher force under the hoof with the greater hoof angle, which was unexpected. Research has suggested that the hoof with the lower hoof angle would experience more loading. This pilot study does suggest that horses with forelimb asymmetries may experience differences in hoof loading.

Equine behaviour analysis in a bilateral lameness model as a precursor to automated detection

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The patterning of exploratory behaviours manifested by certain animals has been associated with their health status. It is not clear how this patterning is expressed in equines. Our long-term research goal is to automatically detect behaviours using wearable sensors to sensitively and objectively monitor training loads and injury risk in equine athletes. The first step in this process is to ascertain what pertinent behaviours need to be included in such an analytical model, before training the sensor data for automatic behaviour detection. Here we describe this first step using a bilateral lameness model to effect pain-related behaviour change. Bilateral synovitis of the intercarpal joints was induced in seven equines using lipopolysaccharide (0.25 ng) at time zero. Animals were confined to stables and monitored intermittently over seven days by video cameras and four wearable sensors. This preliminary analysis focuses on annotated behaviours across the first 0-12h period. Fifteen behaviours were assigned an 'exploratory'/'non-exploratory' code. We computed 'durations of non-exploratory behaviours' (DNEB) (e.g. quiet standing, lying) across the 0-12h period. Visual inspection indicated moderate to severe lameness from 4-8h. Our analysis revealed that 5 out of 7 animals demonstrated a consistent trend of increased DNEB in the 4-8h period compared to 0-4 h, despite differing baseline magnitudes. Further analysis is underway regarding the horses' recovery trajectory, moving to more subtle pain presentations or pain-free states. This will determine whether DNEB is a useful and sensitive indicator of bilateral lameness, suitable for 'training' the sensor data that was concurrently captured in this experiment.

Effects of tendon injury on uninjured regional tendons in the distal limb

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Following tendon injury, little is known about the potential for pathology to develop in regional tendons from overloading or altered function. This study aimed to investigate gene expression and histopathological changes that occur within the deep digital flexor tendon (DDFT) after injury to the superficial digital flexor tendon (SDFT), and the flexor tendons (SDFT and DDFT) after injury to the extensor tendons. Three groups (n=6) of merino wethers (n=18) underwent either partial transection of the SDFT, complete transection of the extensor tendons or were non-operated controls. Operated sheep received one dose of flunixen meglumine and ceftiofur sodium prior to surgery. Eight weeks after surgery, tendons were harvested and regionally sampled for gene expression (real time PCR) and histologic analysis. Transection of the SDFT resulted in increased expression of collagen III, versican, biglycan and lumican ($P<0.026$) within the DDFT. There was no effect of transecting the extensor tendons on the expression of any gene tested in either the SDFT or the DDFT. The DDFT had elevated histopathology scores induced by transection of the SDFT eight weeks previously. There were no histological differences in either the SDFT or DDFT after transection of the extensor tendons. The limitation of this study is the surgical model of tendinopathy which may not correlate with clinically observed overuse injuries. Transection of the SDFT results in a mild, subclinical tendinopathy within the DDFT with potential implications on treatment and rehabilitation of SDFT injuries. Injury to the extensor tendons had no effect on the SDFT or DDFT.

Regional variation in gene expression in the normal ovine trochlear ridge

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Pathology of the femoral trochlear ridges is commonly reported in large animals and the trochlear ridge is often the site for evaluating cartilage repair techniques. However, little is known about the normal cellular activity of cartilage in this region. This study aimed to profile the expression of 24 genes from normal cartilage of the medial trochlear ridge using a sheep model. Six stifle joints from three, two year-old, merino sheep were sampled. Full-thickness cartilage samples (to the level of the subchondral bone) were collected from three sites along medial trochlear ridge from proximal to distal, and analysed using PCR. Mixed regression modelling of the PCR data was performed to determine effects of region and position on gene expression. Expression of aggrecan, collagen 2A1 and SOX9 decreased from proximal to distal, while decorin, lumican, and a disintegrin and metalloproteinase with thrombospondin motifs-4 (ADAMTS4) increased. Versican, collagen 1A1, lumican, ADAMTS5, MMP1, MMP2 and MMP3 expression were lower centrally compared to the peripheral zones, while collagen 2A1 expression was higher centrally. Cartilage gene expression varied significantly throughout the trochlear ridge. This may be secondary to altered weight bearing, patellar pressure, or differing local cellular density. This should be considered when designing *in vivo* cartilage studies so locations for sampling or studying treatment effects using gene expression are standardized in ovine models. Further studies are warranted to determine whether these gene expression differences in the medial trochlear ridge of the stifle occur in other species and whether these differences have any impact on tissue structure and function.

A novel tendon autograft for cartilage resurfacing: an ovine model

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Recent studies have identified that partially transected flexor tendon develops cartilage-like characteristics in early healing which may have potential as an autograft for cartilage repair. We hypothesized that pathological tendon would provide a superior cartilage grafting material compared to normal tendon or no graft. Twenty-one sheep were divided into three groups. Six sheep underwent a partial transection of the hindlimb superficial digital flexor tendon (SDFT). Eight weeks later 18 sheep had chondral lesions created in the trochlear groove. Three sheep were designated non-operated controls. Sheep were treated with either no graft, a normal SDFT graft, or pathological SDFT graft. Tissues were harvested 6 months later for histological and gene expression analysis (PCR). Gene expression data were analysed using mixed regression modelling. A Kruskal-Wallis analysis followed by a Mann-Whitney U test was utilized for histology ($P<0.05$). Grossly, normal tendon graft showed reasonable integration, with 4 of 6 medial defects filled with cartilage-like material. In non-grafted and pathological tendon grafted joints, defects were largely devoid of tissue. Histologically pathological SDFT grafts performed worse than normal tendon grafts and non-grafted lesions. Stifles with normal tendon or pathological tendon grafts had higher gene expression for some collagens, proteoglycans and metalloproteinases and higher synovial collagen, metalloproteinase and IL-8 expression than non-grafted joints. Although histological and gene expression differences are observed at least until 6 months post-operatively, normal tendon shows some potential as an autograft for cartilage lesions. More studies are warranted to assess graft performance in the longer term.

Characterization of correlations between equine hoof anatomy and midstance biomechanics at walk

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Understanding the relationship between hoof biomechanics and anatomy in healthy hooves is imperative before this information can be applied to diagnose hoof pathologies. Variations in hoof anatomy (e.g. toe or heel angle/length) may alter stress distribution, however, experimental studies are scarce. Our objective was to investigate the correlation between midstance hoof kinetics and anatomy in sport horses. Nine unshod athletic horses were walked over a pressure plate and average force (F), contact pressure (CP) and contact area (CA) were recorded. Using digital radiography and digital pictures, 55 variables of internal and external anatomy of the hoof were measured. Correlations between biomechanical and anatomical measurements were investigated using Pearson's rank correlation coefficient. P-values≤0.05 and r-values≥0.5 were considered significant. Steeper/more upright toe angles correlated to lower CA values (r=-0.71). Several heel height measurements were negatively correlated with F on the toe (0.63≤r≤-0.58) suggesting an obvious trend. Larger medial wall angle was correlated with higher F, CP and CA on the medial aspect of the hoof (-0.68≤r≤-0.58. Measurements of the dorsal hoof wall thickness and length/width of the distal phalanx (P3) showed correlations with F and CP (0.5≤r≤0.72) while the height of the P3 was negatively corelated with CA (r=0.72). Patterns of correlations confirmed the relationship between hoof anatomy and hoof biomechanics at walk, signifying the importance of hoof anatomy. A better understanding of such impact on hoof pathologies warrants further research.

Electromyographic changes in superficial forelimb muscles in fatigued Thoroughbred horses

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We previously reported that the integrated-electromyography (iEMG) of major hindlimb muscles decreased with fatigue in horses. However, little is known about EMG changes in forelimb muscles. The objective of this study was to quantify fatigue-induced EMG changes in superficial forelimb muscles in horses on a treadmill. Six healthy Thoroughbred horses were used for surface EMG recording of the M. splenius (Sp), brachiocephalicus (Br), infraspinatus (Inf) and deltoid (Del). Horses galloped on a treadmill inclined to 3% at a constant speed (12.7-13.7 m/s) to make them fatigued after approximately 5 minutes. Before and after this exercise they trotted at 3.5 m/s. Stride frequency (SF), iEMG for a stride and median frequency (MF) of muscle discharge were calculated every 30 s. These parameters were compared at the start and end of galloping for the leading and trailing limbs, and when trotting before and after the fatigue run using a paired t-test. SF significantly decreased at the gallop ($P<0.01$), whereas it did not change at the trot. iEMG of Sp and Br in both leading and trailing limbs at the gallop and those of both left and right sides at the trot significantly decreased with fatigue ($P<0.05$), whereas that of Inf and Del did not change at either gallop nor trot. No changes were observed in MF in any muscles with fatigue. In conclusion, fatigue decreases iEMG of Sp and Br, suggesting that the activity of these muscles is associated with maintenance of speed.

A coupled biomechanical-SPH model of whole-body equine locomotion over two track surfaces

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Racetrack surface properties such as the stiffness, yield stress and spatial distribution of track surface particles affect the loading applied to the limbs of galloping horses. We present an *in silico* model of the racetrack response, coupled with a whole-body model of the horse to estimate hoof impact, tendon and joint loading. Three Thoroughbred horses galloped on dirt and synthetic tracks at 16-18 m/s. Eighty-six markers were used to measure kinematics using 22 motion capture cameras (Vicon Motion Systems, UK). A track testing device (TTD) measured the track surface mechanical properties. Joint angles and centre-of-mass translations were calculated for each trial using custom optimisation-driven inverse kinematics code together with subject-specific skeletal models comprised 42 joints and 43 body segments. Body-segment inertial properties were scaled from published regression tables. A three-dimensional, elastoplastic, smoothed particle hydrodynamics (SPH) model of the track surface was calibrated using the TTD measurements. Dynamic simulations of each trial were developed using the subject-specific and track-specific models. Centre-of-mass rotations and translations and joint angles of the distal limbs were predicted. Model and experimental results were compared to evaluate the model accuracy. Distal net joint torques, muscle forces, and joint contact forces were larger for the dirt track than the synthetic track. Intra-subject differences in muscle and joint forces correlated to differences in gait kinematics. Use of the model to investigate ground surface changes on joint loading was demonstrated using a range of stiffness and plastic material model parameters to understand their effects on kinematics and internal forces.

Radiographic assessment of the equine carpus under incremental loads

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Non-physiologic loading of the carpal bones is believed to result in osteochondral fractures, ligament rupture and axial instability in the equine forelimb; however, the mechanism of carpal damage due to non-physiologic loading of the carpus is largely unknown. To investigate carpal stability (alignment and positions of carpal bones) under load, 24 equine cadaver limbs (aged 10.7±4.2 years) transected at the radial midshaft were vertically loaded into a commercial press and serially radiographed under a range of incremental loads signified by a 10° decrease in the dorsal fetlock angle (DFA) from 160 to 110° (DFA160 to DFA110) using the jacking system of the press. Six previously described carpal parameters were measured from the radiographs. As loading increased from DFA160 to DFA110 there was a progressive significant increase in third carpal bone palmar facet angle from 86.46±2.54 to 88.60±2.51°, but a decrease in dorsal carpal angle (from 173.03±3.47 to 159.65±4.09°); medial carpal angle (from 186.31±1.90 to 184.61±2.26°), and groove diameter of the Cr-Ci intercarpal ligament (GD.Cr-Ci ICL: from 9.35±1.20 to 8.83±1.13°). No significant differences were observed for distal radial slope carpal angle and intermediate carpal bone proximal tuberosity-radial angle. In conclusion, increased load on the carpus produced carpal hyperextension with measurable radiographic changes in the position and alignment of the carpal bones. The small amount of stretch (strain) on the Cr-Ci ICL during loading, indicated by the decrease in width of GD.Cr-Ci ICL, may facilitate absorption and redistribution of concussion forces within the carpal joint during loading thereby providing a useful mechanism to minimise carpal damage.

The effects of arena surface on fore- and hind- limb kinematics of show jumping horses at take-off

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Fetlock angle and hoof translations were compared during take-off for a jump between measured arena surfaces. Forelimb and hindlimb fetlock and hoof kinematic data during stance phase were acquired from three Warmblood horses during take-off over a 1.1 m oxer jump on one dirt surface and one synthetic surface. Fetlock angle and vertical and horizontal hoof translations in the surface were acquired using calibrated 2D video capture at 500 Hz and compared between surfaces using mixed model analyses of covariance. Surface mechanical properties were measured using vertical impact and quasistatic horizontal shear mechanical tests. Forelimb fetlock extension at heelstrike was 2.3° greater (P=0.046) on the dirt surface. Hindlimb fetlock extension at heelstrike was 2.2° greater (P=0.006) and maximum fetlock extension was 2.2° greater (P=0.014) on the synthetic surface. Forelimb and hindlimb hoof translations differed between surfaces during support (P<0.007). Forelimb toe and heel positions displaced 0.41 and 0.09 cm, respectively, dorsally on the dirt surface but 0.20 and 0.44 cm, respectively, palmarly on the synthetic surface. Hindlimb toe and heel positions displaced palmarly on both surfaces but displacements were 0.65 and 0.77 cm, respectively, greater on the synthetic surface. Surface peak vertical impact force, stiffness, and loading rate were 37, 217, and 228% greater, respectively, on the dirt surface (p-values<0.001). Surface vertical impact rebound rate was 141% greater, but marginally insignificant (P=0.079), on the synthetic surface. The dirt surface cushion had lower angle of shear resistance (28°) and lower apparent cohesion (4,007 N/m^2) than the synthetic surface cushion (39°, 5,294 N/m^2).

A preliminary AnyBody model of the equine forelimb for motion simulations

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The physical properties of racing surfaces are associated with injuries in horses. The focus of our research is to test the effects of variable surface stiffness and consistency on discrete strains in the suspensory apparatus and ligaments at the distal joints. A preliminary AnyBody model has been developed to test the limb's responses to varying surface properties. CT renderings of the bony elements of a Thoroughbred forelimb formed the skeleton of the model in AnyBody® and joints were modelled with six rotational degrees of freedom. In this preliminary test of the model, a Thoroughbred horse had reflective spherical markers applied in triads to the forelimb segments, and trotted in front of a 6-camera Qualisys system to collect kinematic data at 200 Hz. The pastern was modelled as a single segment with markers on the proximal phalanx. Sagittal plane joint angular displacements were compared using Visual3D and the AnyBody model animated with the kinematic data. In the stance phase, sagittal range of motion of the coffin joint varied by 9° (Visual3D: 31 to -9°; AnyBody: 26 to -6°); the fetlock joint varied by 20° (Visual3D: 5 to -75°; AnyBody: -15 to -75°); the carpal joint varied by 3° (Visual3D: -84 to -15°; AnyBody: -87 to -15°); the elbow joint varied by 4° (Visual3D: 94 to 49°; AnyBody: 98 to 49°); the shoulder joint varied by 1° (Visual3D: -75 to -88°; AnyBody: -75 to -89°). The angles calculated in both systems are sensitive to variations and discrepancies in joint axis locations and orientations.

The effect of ground surface on head and pelvis displacement at the trot

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Ground surface compliance can alter the horse's gait and the risk for musculoskeletal injury. The objective of this paper was to quantify changes in gait associated with surface and ridden vs unridden. Using a randomised split plot data were collected from 8 sport horses in regular work (6-23 years old, 156-166 cm height) when led 'in-hand' (unridden) on 3 ground surfaces (asphalt [asphalt], packed dirt[hard], sand mix arena [soft]), and 2 ground surfaces (hard and soft) when ridden at the trot (~3 m/s). Each horse was fitted with an inertial sensor system (Equinosis LLC) and data captured for 5 runs of 12 consecutive strides in a straight line. Velocity data were captured using a Polar GPS /HR system. Surface compliance was quantified using a penetrometer and a clegg hammer. Accelerometer data (head and pelvis) were captured at 200 Hz and exported to R studio for analysis. Data were filtered using wavelet analysis to remove frequencies above 12.5 Hz, and displacement calculated. Data were examined using a General Linear Model. Unridden, the horses trotted slower on the asphalt than on hard or soft surfaces (2.93 (0.33) vs 3.81 (0.36) vs 3.13 (0.31) m/s, $P<0.001$ respectively), with no difference in stride frequency. Vertical head displacement and pelvis displacement was least on asphalt and greatest on soft surface (124 vs 129 vs 138 mm, $P<0.001$ respectively and 161 vs 164 vs 179 mm, $P<0.001$ respectively). Ridden there was no difference in velocity, but a greater stride frequency on the hard surface vs soft (1.40 (0.08) vs 1.38 (0.27) strides/s, $P<0.001$). Head [122.9 (37.1) vs 115.0 (30.2) mm, $P<0.001$] and pelvic vertical displacement [165.1 (18.3) vs 152.2 (22.3) mm, $P<0.001$], were greater on the soft vs hard surface. The horses' vertical displacement reduced with less compliant surfaces. Riders regulated velocity, resulting in increased stride frequency on less compliant surfaces.

Pilot study examining centre of pressure in the forelimbs of Thoroughbred racehorses

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The development of sensitive techniques to monitor a horse's response to race training may help reduce the risk of injury. The objective of this study was to examine the effect of age and stage of training on the standing centre of pressure (COP) in Thoroughbred racehorses. COP data of the forelimbs were collected from 34 Thoroughbreds (5 years old, IQR 4-6) in race training using a 0.5 m^2 Footscan pressure plate. Workload data were collected from the trainer and from a central database (www.nzracing.co.nz). COP data were collected at 15 Hz using the Footscan stability software. Data were filtered and statistical analysis performed (regression and non-parametric analysis of variance) within R Studio (v3.1.1). The median number of months in training was 3 (IQR 2-5) months and the median number of training gallops during this preparation was 11 (IQR 3-20). There was a right limb bias in mean limb load (747 [IQR 661-904] vs 655, [559-801] N, $P<0.001$). The mean COP frequency was 0.3±0.08 Hz, amplitude (minima to maxima) 16.9±12.3 mm and velocity of COP 1.1±1 mm/s. There was a linear relationship between amplitude (mm) and total COP path (mm), $R^2=0.58$, $P<0.05$ and amplitude and velocity ($R^2=0.59$, $P<0.05$). The relationship between amplitude and frequency was moderate and curvilinear ($R^2=0.28$, $P<0.05$). There was no significant association of age within any model. Horses with >10 training gallops had smaller COP amplitude (10.5, [IQR 7.2-17.4] vs 21.7, [11.6-29.3] mm, $P<0.05$). There was no difference in median velocity between the groups. The consistent right limb loading bias indicates this may be present irrespective of training load. The reduced amplitude may be associated with greater muscular tone, or horse experience.

Effect of pre-exercise massage on stride length of the forelimb in horses

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Massage has its effects on muscle fibres by initiating compliancy and elongation resulting in enhanced extensibility. This study assessed whether certain massage techniques applied to specific forelimb extensor muscles would increase stride length at trot. Five Thoroughbred geldings (age 4-22 yr) received either a pre-exercise massage or no massage in a randomized crossover design. Prior to the start of the study, all horses participated in a college lesson horse program. Forelimb stride length was measured with reflective markers attached to the skin over five standard positions. Thermal imaging was recorded of the left and right trapezius, serratus ventralis, latissimus dorsi, triceps, and ascending pectoral muscle groups at pre-massage, pre-trot, and post trot. The massage protocol included one minute effleurage, 10 minute compression, and 10 minute friction. Horses were filmed using a 125 Hz camera placed approximately 35 m from the trotting track. The horses trotted at a minimum of 3.8 m/s for 52 meters. Ten consecutive strides were digitized using the Quintic Biomechanic Video Analysis Software System for evaluation of stride length. Data were analysed using PROC GLM of SAS. Results are presented as mean±SD. Horses were trotted at 4.19±0.2 m/s. Stride length increased 2.8±0.2 cm with massage ($P<0.05$). Non-massage thermal muscle temperatures increased 0.47 °C ($P<0.05$). Muscle temperatures decreased 0.34 °C with massage ($P<0.05$). Results of this experiment support the hypothesis that pre-exercise massage increases stride length of the equine forelimb. Further research is needed to evaluate the physiological effects of specific massage techniques on muscle fibres.

Effects of boots on movements in trot of Icelandic horses on a treadmill

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In Icelandic horses, boots are commonly used as weights on the front limbs to correct beat, and increase swing time and stride length in trot. The aim of this study was to determine if 240 g boots on the front limbs, compared to no boots, had an effect on stride parameters at trot in Icelandic horses on a treadmill. The study had a crossover design with eight horses, two treatments (no boots, 240 gr boots) and was performed on two days on a high-speed treadmill (Säto, Sweden). Horses performed a standardized warmup (5 min walk 1.6 m/s, and 5 min trot 4.5 m/s), and an exercise test (10 min trot, 4.5 m/s) with 1 min at walk (1.6 m/s) in the end. A high-speed camera (200 fps) was used to record during 20 s in the sixth minute of the exercise test; 10 strides were measured. Stride length, stride frequency, duty factor and beat were calculated from timing of hoof-on and hoof-off. Results were analysed with a paired t-test in SAS by pooling data for right and left limbs/diagonals ($P<0.05$). Duty factor of front limbs was shorter with boots than without boots (38.0±1.8 vs 38.9±1.8%; $P<0.05$) and swing time increased proportionally. Stride length, stride frequency and beat were not different between treatments. It was concluded that 240 g boots can increase swing-time proportionally of the front limbs, which is a valuable trait in competitions and breeding assessments of Icelandic horses.

Routine hoof trimming and changes in regional forehoof biomechanics at midstance

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Uneven hooves may predispose horses to musculoskeletal injuries. Hoof balance has traditionally been achieved by hoof trimming and farriery, which rely heavily on visual examination of the hoof. Evidence-based research may improve our understanding and provide data that are more objective. Our objective was to measure and compare force (F), contact area (CA), contact pressure (CP) and peak contact pressure (PCP) of the dorsal vs palmar and medial vs lateral regions of the front hooves. Ten sound athletic horses were led at walk over a calibrated pressure plate before and after routine hoof trimming. Distribution of the F, CA, CP and PCP in medio-lateral and toe-palmar regions was examined before and after trimming. P-values ≤0.05 were considered significant. The CP ($P=0.012$) and PCP ($P=0.013$) at the toe (dorsal) region significantly increased post-trimming. The pre-trim dorsal force was significantly greater than the palmar force, and this difference remained significant after trimming ($P<0.0001$). Medial force increased about 25% after trimming, but the change was not statistically significant ($P=0.07$). While many horses land heel first and/or favour the lateral aspect of the hoof; we found that F and CP shift to the dorsal region as the stride approaches midstance; such shift contributes to hoof stability at midstance. Asymmetrical force and pressure distribution might transfer the excess stress to more sensitive structures within the hoof or towards more proximal structures, subsequently causing injuries. Our results provide a benchmark for the influence of trimming on medio-lateral and toe-heel kinetics, assisting farriers and veterinarians to better assess hoof balance.

Objectively assessed trotting symmetry of Warmblood horses admitted for prepurchase examination

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Increased use of objective locomotion analysis in clinical practice raises the question if it could be used in connection to prepurchase examinations (PPE), which would require understanding of asymmetry levels in horses admitted for PPE. Therefore, this study aimed to objectively assess the locomotor symmetry status of particularly that group of horses. We hypothesized that their symmetry values would fall within the reference values reported earlier to discriminate between lame and non-lame horses. The pilot group consisted of 14 high-class Warmblood horses (mean 8.8 years, range 6-13) that passed their PPE. They were assessed while trotting on a groomed soft surface on a straight line using a 20-camera optical motion capture (OMC) system (Qhorse®). Mean differences of the absolute minimal vertical displacement (|MinDiff|) of head (n=14 strides) and pelvis (n=20 strides) were 8.8 mm (range 1.2-14.8 mm) and 3.0 mm (0.3-7.8 mm), while |MaxDiff| of head and pelvis were 6.0 mm (range 0.6-19.8 mm) and 3.1 mm (range 0.7-6.8 mm). Mean symmetry values of this group of horses that passed their PPE, collected using an OMC camera system, showed a similar range of asymmetry as reported previously using a different, IMU sensor system (6 mm for the head and 3 mm for the pelvis) on non-lame horses. However, ranges show that in individual cases the degree of asymmetry may be considerably larger. Thus, symmetry of gait is not an absolute decisive factor on its own in PPE, and other factors, consciously or unconsciously, are taken into account as compensating for a greater degree of asymmetry.

Applied physiology – New technology in equine exercise physiology

Muscle OXPHOS capacities in endurance horses predict racing performance

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Aerobic ATP production by the mitochondrial oxidative phosphorylation system (OXPHOS) provides most of the energy for muscle contraction during endurance exercise. We hypothesized that OXPHOS capacity of individuals is predictive of their performance in endurance racing. Muscle OXPHOS capacity was measured by high-resolution respirometry (HRR) in permeabilized triceps brachii fibres obtained by microbiopsy (<20 mg). This advanced technology provides a quantitative measurement of mitochondrial respiration. Muscle OXPHOS capacity was determined in ten trained horses belonging to the national endurance team of France. One month after this study, all horses participated to a 160 km endurance race (CEIO**** Compiegne, France). All but one horse completed the ride and out of 47 participants, they reached the 1st, 2nd, 3rd, 4th, 7th, 9th, 11th, 16th and 19th place. Linear regression analyses indicated that OXPHOS capacities were significantly correlated to finishing place (the first horses had the highest OXPHOS capacities; R^2=0.76; P<0.001) and speed (the fastest horses had the highest OXPHOS capacities; R^2=0.74; P<0.0001). In addition, OXPHOS was strongly predictive of the ranking for the four first places. Interestingly, the highest OXPHOS capacity was found in the horse who won the prize of the best-conditioned endurance horse the next year at the World Equestrian Games in Kentucky. This study suggests the practical value of HRR applied to microbiopsies to determine athletic capacities in endurance horses.

Assessing the association of speed and stride parameters via GPS technology in Thoroughbred racing

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Distance and speed galloped by racehorses has been used to assess the risk of musculoskeletal injury (MSI). It is however, the number of strides that determines the number of skeletal loading cycles and therefore the extent to which fatigue damage accumulates. Although speed and stride characteristics (count, length, duration) are related, the usefulness of using speed and distance as a proxy for stride count is unknown. We performed a retrospective analysis of stride characteristics in flat race starts (n=25,040) between 2011 and 2016, via GPS and inertial sensors (StrideMaster®). Sectional data from three stages of each race was analysed to determine if speed predicted stride parameters. Univariable screening of predictor variables was performed, followed by multivariable regression via backwards stepwise elimination adjusting for clustering at the horse level (P<0.05). Mean (±SD) stride length, count, duration and speed were 7.08±0.39 m, 28.32±1.56 strides/200 m, 0.43±0.02 s/stride and 16.63 m/s for all starts and sectionals, with stride length decreasing, stride count increasing and speed reducing with race progression (P<0.001). Speed correlated with stride count in the early (R^2=0.43) and mid (R^2=0.36) stages of the race, with the strongest correlation in the final sectional (R^2=0.54). In multivariable analysis, males, racetrack, greater race distances, better finishing positions, and firmer track surfaces were associated with fewer strides per sectional. There was substantial inter-horse variation in stride parameters and speed predicted only about half this variation. To better assess the impact of racing history on MSI risk, stride variables should be considered.

Smartphone data for stride length and velocity in 971 exercise sessions by racehorses in training

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The relationship between stride length (SL) and velocity (V) in Thoroughbred racehorses in training was investigated using data generated in real training situations. Stride length and velocity in 41 Thoroughbred racehorses in training were measured using a novel smartphone application (KER ClockIt RACE™) recording GPS position, V and accelerometer data. Data were acquired during routine training sessions (n=971) undertaken by 41 horses at 12 different training centres located throughout the world. Data were processed using filtering and Fourier Transforms to remove noise and determine SL. The mean V of the fastest 200 m track sectional run by each horse was 15.8 m/s. Mean SL during fastest 200 m track sectional was 6.8 m (range 5.8-7.6 m). Data at V below 5.5 m/s and data in which the horse was decelerating were discarded for SL analysis. Valid data for all sessions run by each horse were combined, and visualised using SL vs V scatter plots. Regression analysis confirmed a linear relationship in all horses between SL and speeds of 5.5-11.1 m/s (P<0.01). At speeds above 11.1 m/s a quadratic (or non-linear) relationship between SL and V was found to be present with a statistical significance of P<0.01 in 29 of 41 horses. Horses in the linear group increased speed by increasing SL whilst maintaining a constant stride frequency. Horse in the non-linear group increased speed above 11.1 m/s by increasing both SL and stride frequency. Further research is required to determine if the two groups of horses have different racing characteristics and whether SL analysis has application in early injury detection. There is potential for large-scale population studies using this novel data acquisition methodology.

Respiratory muscle training for the treatment of dynamic upper airway collapse in racehorses

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Respiratory muscle training (RMT) is used in humans for the treatment of upper airway collapse. A bespoke equine mask and incremental training protocol were previously developed to provide resistance during inspiration, with the aim of improving upper respiratory muscle strength. The main objective of this study was to assess the change in dynamic upper airway collapse following a period of RMT. Thoroughbred racehorses with a diagnosis of upper airway collapse during exercising endoscopy were recruited. Horses undertook RMT 5-6 days/week while continuing normal ridden exercise. Repeat exercising endoscopy and a similar exercise test were performed at the end of the training period. The only diagnosis not recruited was complete left arytenoid cartilage collapse. Video recordings were graded by three blinded, board-certified reviewers and airway function scores were compared using a Wilcoxon Signed-Rank Test. Eight Thoroughbred racehorses successfully completed the RMT program. Diagnosis at recruitment was often multifactorial including; vocal fold collapse (VFC), medial deviation of the aryepiglottic folds (MDAF), palatal instability (PI), and dorsal displacement of the soft palate (DDSP). There was a significant improvement in objective video-endoscopy scores following RMT (P=0.012); including VFC (3/5 horses), MDAF (4/6 horses), PI (4/6 horses) and resolution of DDSP (5/6 horses). The mean peak training resistance was 37 cm H_2O (25-50 cm H_2O). The mean number of RMT days prior to reassessment was 39 (21-62). All horses tolerated the RMT well. RMT can lead to improvement in upper airway function and may become a viable alternative to surgical treatment.

The influence of whole body vibration on bone mineral content in the stalled horse

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Whole body vibration (WBV) is an emerging therapy that has shown success in maintaining bone density in humans and horses. As stalling has shown deleterious effects on bone, this study aimed to evaluate the effect of WBV on bone mineral content (BMC) in stalled, healthy horses. Twelve mature mixed breed horses were randomly assigned to either control (n=6) or vibration (VIB, n=6). All horses were stalled for a 28-d period, and VIB horses stood on a WBV platform (Equivibe, Malcolm, Nebraska, USA) at 50 Hz for 45 min 5 d per week. Radiographs were taken of the left third metacarpal on days 0 and 28 for determination of cortical BMC using radiographic bone aluminium equivalence (RBAE). Statistical analysis was performed using a mixed model ANOVA in SAS 9.4. Change in BMC was not different due to treatment in medial (P=0.42), lateral (P=0.20), dorsal (P=0.07), or palmar (P=0.11) cortices. Contrary to previous research, BMC increased in both groups over time in all cortices (P<0.05). This study was unable to demonstrate a decrease in BMC attributable to stalling, though previous studies have reported changes in 14 d or less. It may also be that stalling may be less detrimental to BMC in the mature horse. Still, WBV did not increase BMC in any bone cortex over control. The results of this study suggest no influence of WBV on BMC in stalled, heathy horses if stalling-related bone loss is not present.

Evaluation of bronchoconstriction using electrical impedance tomography (EIT) in horses

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Bronchoconstriction during and after exercise is difficult to demonstrate in mild equine asthma. Electrical impedance tomography (EIT), a novel non-invasive imaging technique, may be conducive to use in exercising horses. The technique involves measuring impedance changes over a breathing cycle as measured by 32 electrodes mounted on a belt placed around the thorax. The aim of the study was to evaluate if bronchoconstriction after histamine provocation could be detected using regional and global respiratory gas flow signals calculated from the EIT impedance change and to compare it to delta flow (Δflow) measured by open plethysmography (Open Pleth™). Six healthy sedated (detomidine 0.01 mg/kg IV) horses were concurrently fitted with both devices. At baseline saline was nebulized into the mask. Thereafter increasing concentrations of histamine were nebulized until bronchoconstriction was clinically confirmed and Δflow increased to greater than 50%. Data from both methodologies were recorded concurrently. Regional EIT-based peak expiratory flow was evaluated by calculating the first derivative of the EIT signal. Global (GFEIT) and regional flow was summarized for the dorsal (DFEIT) and the ventral (VFEIT) lung area over 10 breaths. Consistent with bronchoconstriction, a significant increase was seen in Δflow (P=0.025). GFEIT (P=0.003) and VFEIT (P=0.03) decreased significantly during bronchoconstriction. VFEIT decreased in 4/6 horses, while DFEIT decreased in 3/6 horses. Bronchoconstriction can be verified by evaluating the EIT gas flow signal after histamine provocation and may allow this modality to be used in horses comparing measurements before and after exercise.

Applied physiology – Drugs in equestrian sport and exercise

Efficacy of firocoxib and acetaminophen combined therapy in a reversible model of foot pain

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Firocoxib and acetaminophen have shown promise in the relief of pain in horses. Using a model of equine foot pain, the objective was to test the hypotheses that a combination of IV firocoxib and oral acetaminophen is (1) more efficacious in alleviating lameness than placebo and (2) comparable in efficacy to IV phenylbutazone. One hour after lameness induction using adjustable heart bar shoes, 8 healthy horses (7 TB, 1 WB; mean age 6.5±1.0 years, range 2-to-11 years; 6 geldings, 2 mares) underwent weekly treatments administered by a co-investigator who had no input on heart rate (HR) and lameness score (LS) measurements. Randomized treatments 1 h after lameness induction included isotonic saline placebo (1 ml/45 kg body weight IV); phenylbutazone (4.4 mg/kg IV BID); and firocoxib (loading dosage 0.27 mg/kg IV once) combined with acetaminophen (20 mg/kg PO BID). Another investigator, unaware of treatment assignments, monitored HR and LS every 20 min for 4 h and then hourly through 24 h post-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 $P<0.05$. Post-treatment phenylbutazone HR (3-5, 7, 9-24 h) and LS (0.7-24 h) were lower than placebo ($P<0.05$). Post-treatment firocoxib + acetaminophen HR (2.3, 3-3.7, 9, 11-17, 20-23 h) and LS (1.3-24 h) were lower than placebo ($P<0.05$). It was concluded that firocoxib and acetaminophen combined were more effective than placebo and comparable to phenylbutazone at these dosages.

Effects of a type-5 phosphodiesterase inhibitor on pulmonary artery pressure in race fit horses

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This study was performed to determine the optimal dose and timing of E4021 (EIPHISOL™) to reduce pulmonary artery pressure (PAP) during treadmill exercise. Eight (4 geldings, 4 mares) unfit Standardbreds (4-8 y, ~490 kg) were conditioned for the entire trial. Speed and duration increased weekly until week 12-14, when three treadmill GXT were performed to document stable fitness (VO_{2max}). Two randomized crossover experiments then used simulated race tests (SRT) to determine dose and timing of IV administration of E4021. Experiment 1: no drug (CON-A) or two doses (50 vs 100 mg) and two time points (45 vs 90 min). Experiment 2 (all 90 min): no drug (CON-B); 100 mg (100B); 150 mg; or 200 mg. The SRT comprised a 2-min warm-up, 2-min at 110% VO_{2max}, 2-min recovery. PAP, ECG, VO_2, and VCO_2 were measured continuously and blood (3 ml) collected anaerobically at end of warm-up, 1 and 2 min at high speed, and at the end of recovery to measure $PpaO_2$, $PpaCO_2$, pH, sO_2, $[Na^+]$, $[K^+]$, $[Ca^{++}]$, [lactate], [glucose], [haemoglobin], and PCV. Analysis included repeated measures ANOVA, Dunnett's and Tukey tests ($P<0.05$). The major finding was that the 100 mg dose administered 90 min before exercise resulted in the lowest PA pressure ($P<0.05$). There were no differences in PAP during the 100 vs 150 vs 200 mg trials. E4021 did not alter markers of aerobic or anaerobic performance. The ~30 mmHg lower PAP with 100 mg at 90 min represents a clinically significant effect.

EIPH in horses: effects of furosemide on pulmonary transmural pressure

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Maximal intensity exercise induces pulmonary capillary stress failure secondary to excessive transmural pressure (TransP). Furosemide decreases EIPH severity and pulmonary arterial pressure (PAP). The aim of this study was to assess the effects of furosemide on TransP during supramaximal exercise. Five racing Thoroughbreds with consistent EIPH participated in a controlled trial using a treadmill exercise test to fatigue at 120%VO_{2max}. Horses exercised before and 4 h after furosemide injection (0.5 mg/kg IV). Vascular catheters measured left ventricular pressure (LV), PAP, and pulmonary capillary wedge pressure (PACWedge, without right ventricle pressure recording) during exercise. A balloon-tip oesophageal catheter measured transpulmonary pressure (P_L). Mean TransP ($\overline{TransP}$) and maximal TransP ($TransP_{max}$) were calculated as: $\overline{TransP} = P_{Lmin} - meanPAP$ (averaged), and $TransP_{max} = P_{Lmin} - PAP_{max}$ (averaged). Endoscopic EIPH scoring (EIPHscore) and BAL erythrocyte count (BALrbc) were performed 1 h post-exercise. Differences and associations were tested using a Wilcoxon signed rank and a Spearman test, respectively ($P<0.05$). EIPHscore ($P=0.03$) and BALrbc ($P=0.02$) decreased after furosemide. The LV ($P=0.015$), PAP ($P=0.03$) and PACWedge ($P=0.09$) decreased during exercise after furosemide administration. There was a strong correlation between PACWedge and EIPHscore ($r^2=0.63$; $P=0.05$) and BALrbc ($r^2=0.65$; $P=0.04$). The $\overline{TransP}$ and $TransP_{max}$ decreased significantly ($P=0.03$ both) after furosemide administration (pre-furosemide $\overline{TransP}$=152 mmHg (120-242); post-furosemide $\overline{TransP}$=122 mmHg (78-145); pre-furosemide $TransP_{max}$=166 mmHg (160-206); post-furosemide $TransP_{max}$=106 mmHg (92-157). Furosemide administration decreased EIPH as well as $\overline{TransP}$ and $TransP_{max}$. However, these pressure values stayed above the documented pulmonary capillaries stress failure threshold, and EIPH was still observed.

Urine and plasma elimination of dexamethasone sodium phosphate following a single nebulization

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The objective of this study was to establish the time for a single dose of dexamethasone sodium phosphate (DSP), administered to adult horses using a FlexinebE2® nebulizer, to be eliminated from urine and blood. Six Standardbred mares were given a single dose of 0.04 mg/kg DSP diluted in 0.9% NaCl and administered as an aerosol using a FlexinebE2® nebulizer. Blood samples (0 [baseline], 2, 4, 6, 8, 10, 12, 24, 32, 48, 72 and 96 h) and urine samples (0 [baseline], 1, 4, 8, 24, 32, 48, 72 and 96 h) were collected for analysis using mass spectrometry at an official Forensic Racing Laboratory. Plasma concentrations reached t_{max} at the earliest collection point (2 h) after nebulization and ranged from 0.6 to 1.8 ng/ml. Dexamethasone was no longer detectable in the blood of any of the horses at 48 h. The t_{max} in urine was reached at the earliest collection point (1 h) after nebulization and ranged from 3.2 to 23.8 ng/ml. Dexamethasone was no longer present in urine at 72 h in 5 horses while detectable levels (0.1 ng/ml) were still present at 96 h in one horse. In conclusion, a single dose of 0.04 mg/kg of dexamethasone sodium phosphate administered as an aerosol through a Flexineb E2® mask was no longer detectable in blood at 48 h in all horses tested and was only detectable at a low concentration (0.1 ng/ml) in urine by 72 h in one horse.

30-day oral acetaminophen tolerance in adult horses

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Despite its increasing clinical use in athletic horses, there are no controlled studies of equine acetaminophen tolerance. The objective was to test the hypothesis that acetaminophen administered chronically at an oral dosage 25% higher than that sometimes used in horses would result in measurable hepatotoxicity as seen in humans and other species. Six healthy adult horses were administered 25 mg/kg acetaminophen powder in corn syrup twice daily for 30 days. Three other horses served as negative controls receiving only corn syrup twice daily. Jugular venous blood samples were obtained in EDTA and heparinized vacuum tubes on days 7 and 1 before treatment; treatment days 1, 2, 5, 8, 12, 15, 19, 22, 26 and 30; and days 3 and 7 after treatment ceased. Samples were analysed the same day for complete blood counts and plasma biochemistry concentrations including GLDH, AST, SAP, GGT, CK, BUN, creatinine, TP, glucose, and electrolytes. Repeated measures analysis of variance and *post hoc* Tukey's test were used to identify differences between treatment groups ($P<0.05$). On all sampling days, measured values were within the normal range for the analysing laboratory, and there were no differences between treated and untreated horses. It was concluded that acetaminophen at this oral dosage was well tolerated and was not measurably toxic to the six horses receiving the drug in this manner. It is postulated that the use of 20 mg/kg orally twice daily should be safe for durations of up to 30 days in healthy horses.

Efficacy dynamics of oral vs intravenous phenylbutazone

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Despite widespread use of phenylbutazone (PBZ) in horses, the efficacy dynamics of oral vs intravenous (IV) PBZ have remained poorly documented. The objective was to test the hypothesis that IV PBZ has earlier onset and greater efficacy than oral PBZ. Nine healthy adult horses (mean age 14.4±1.4 years, range 5-21 years; 3 WB, 2 STB, 1 TB, 1 QH, 1 Arabian, 1 crossbred; 2 geldings, 6 mares, 1 spayed mare) had an adjustable heart bar shoe applied to the left front foot. Lameness score (LS) of 4.0/5.0 (modified AAEP scale) was achieved initially in each subject by tightening a set screw against the adjustable heart bar 1 h prior to treatment. Treatments were administered in a randomized fashion so that all treatment order permutations were represented. Treatments included: PBZ (4.4 mg/kg PO SID commercial paste), PBZ (4.4 mg/kg IV SID), and isotonic saline (1 ml/45 kg SID). One blinded investigator measured all HR and LS in the stall every 20 min for the first 5 h and then hourly for an additional 8 h. Repeated measures ANOVA and *post hoc* Tukey's test were used to identify analgesic effects ($P<0.05$). Post-IV HR (2, 3, 7-9, 11 h) and LS (1-12 h) were lower than saline ($P<0.05$). Post-oral HR (9, 12 h) and LS (3, 5-12 h) were lower than saline ($P<0.05$). It was concluded that IV administration of PBZ had earlier onset of efficacy than oral dosing but both were still effectively lowering LS 12 h after dosing.

Effect of terbutaline on potassium balance and fatigue during incremental treadmill exercise

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Anecdotally, pre-exercise administration of β-2 agonists like clenbuterol and terbutaline enhances the performance of racehorses by a mechanism unrelated to ventilation. β-2 agonists drive potassium (K^+) into cells and increase intracellular [K^+] ([iK^+]), and one factor in the development of muscular fatigue is slowing of Na^+-K^+ATPase pump activity and decreased [iK^+]. The aim was to assess the effects of terbutaline (T) on intraerythrocytic [K^+] ($[iK^+]_{rbc}$) and plasma [K^+] in Thoroughbreds horses during treadmill exercise. In a blinded, randomized crossover study (n=5), 0.02 mg/kg SC terbutaline or a placebo (P) were administered 30 min prior to an incremental treadmill (5% slope) test to fatigue. Following a 3 min warm-up (4 m/s), horses exercised at 8, 10, 11, and 12 m/s for one minute or until fatigued. Venous blood for determination of plasma [K^+] and ($[iK^+]_{rbc}$ was obtained before treatment, immediately before exercise, and in the last 10 s of exercise at each speed. Data were analysed by two-way RM ANOVA (significance set at $P<0.075$, due to the small n). Exercise was associated with an increase in plasma [K^+] ($P<0.001$) and decrease in [iK^+] ($P=0.04$). When compared to placebo results, terbutaline decreased [K^+] (8 m/s: 6.1±0.4(P), 5.0±0.6(T); 12 m/s: 7.8±0.6(P), 7.2±0.3(T) mmol/l; $P=0.04$) and increased $[iK^+]_{rbc}$ (8 m/s: 36.3±7.1(P), 50.5±8.8(T); 12 m/s: 29.2±7.9(P), 41.3±4.3(T) mmol/l; $P=0.055$) at 8-12 m/s and increased the run time to fatigue (456±39.0 s(P), 483±37.0 s(T); $P<0.001$). In conclusion, pre-exercise terbutaline administration delays the onset of fatigue, presumably by slowing the exercise-associated decrease in [iK^+]. This might be the basis for improved performance following administration of a β-2 agonist.

Methylprednisolone acetate suppresses intra-articular bone morphogenetic protein expression

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Corticosteroids, such as methylprednisolone acetate (MPA), are potent anti-inflammatory drugs commonly administered to manage equine arthritis. Corticosteroids also suppress articular chondrocyte biosynthesis. Bone morphogenetic proteins (BMPs) are required for articular cartilage homeostasis. We hypothesized that intra-articularly administered corticosteroids suppress synoviocyte and chondrocyte BMP expression and activity. Eighty mg of MPA, a high clinical dose, was injected into one tibiotarsal joint of five healthy adult horses. Saline was injected into contralateral joints. Horses were euthanized seven days later. Articular cartilage and synovium were collected for RNA isolation. Synovial fluid was collected to measure BMP activity. Articular chondrocytes were isolated and maintained as aggregates in serum-free medium. After 48 hours, chondrocytes were exposed to 10^{-10}-10^{-5} M MPA and varying concentrations of BMP-2 protein. The ability of BMP to antagonize MPA's effects on matrix synthesis was assessed by qPCR, collagen type II ELISA and DMMB assessment of sulphated glycosaminoglycans (sGAG). Outcomes were analysed using matched t-tests or ANOVA. *In vivo*, MPA significantly suppressed BMP-2 and -6 expression in chondrocytes and synoviocytes. MPA reduced synovial BMP levels in all horses. *In vitro* effects of MPA were similar. Exogenous BMP-2 protein increased collagen type II and aggrecan mRNA expression 2-3 fold, but restored collagen and sGAG synthesis to only 50% of control values. These results confirm that MPA suppresses endogenous intra-articular BMP activity. Exogenous BMP mitigates this, but does not completely restore biosynthetic activity. These findings are relevant to cases where the objective is to maintain cartilage matrix integrity or stimulate cartilage repair.

Evaluation of cobalt as a performance enhancing drug in fit Standardbred race horses

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Seven healthy, race-fit Standardbreds (4 geldings and 3 mares; 5±3 y; ~490 kg) were used to test the hypothesis that cobalt administration would alter biochemical parameters related to red blood cell production as well as exercise performance. Before receiving any drug treatment, all completed a series of baseline testing including an incremental exercise test (GXT) to measure VO_{2max}, biomarkers of performance, vascular volume as well as concentrations of plasma lactate, and erythropoietin. Drug administration commenced seven days after the pre-dosing GXT. Each horse was administered a sterile solution of cobalt salts (50 mg of Co HCl in10 ml of saline, IV) at 09:00 on three consecutive days via the jugular vein. Blood samples were obtained from the contralateral jugular vein before and at 1, 2, 4 and 24 h after administration. Plasma and blood volume were measured one day after the last dose of cobalt; followed by a post-administration GXT performed the next day. Horses were observed for signs of adverse effects of the cobalt administration (agitation, sweating, increased respiration, etc.). Statistical analysis included repeated measures ANOVA and SNK. Cobalt administration increased (P<0.05) plasma cobalt concentration from a pre-administration mean (± SE) of 1.6±0.6 ppb to 369±28 ppb following three doses of the cobalt solution. There were no changes (P>0.05) in markers of aerobic and anaerobic performance, nor any changes (P>0.05) in plasma EPO concentration, plasma volume, resting blood volume, total blood volume, or estimated red blood cell volume. There were no observed adverse effects.

Effect of furosemide on [H+] across the lung in exercising horses

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Furosemide (Fur) is prescribed for management of EIPH in racehorses. Renal effects of furosemide on plasma hydrogen ion ($[H^+]_p$) are well-known, whereas its effect on $[H^+]_p$ changes across the lung have not been described. The purpose of this study was to determine the effects of independent acid-base variables (PCO_2, [SID], A_{tot}) on $[H^+]_p$ after exhaustive exercise in horses pre-treated with Fur. In a crossover study, six horses were treated with 250 mg of Fur IV or placebo (equal volume of saline solution) four hours before treadmill exercise at 80% VO_{2max}. Arterial and mixed-venous blood were sampled at fatigue. Plasma acid-base variables, as well as whole blood and erythrocyte Na^+, K^+, Cl^-, Lac^- were measured. Contributions of independent variables to $[H^+]_p$, and an individual contribution of strong ions to whole-blood, plasma and erythrocyte [SID] changes across the lung (ΔSID) were calculated using AcidbasicsII. Paired-sample t-test was used for data analysis. Furosemide had no effect on time to exhaustion and had no effect on contributions of PCO_2, [SID] and A_{tot} to $[H^+]_p$ in pulmonary circulation. However, it decreased erythrocyte Cl^- contribution to ΔSID from 5.0±1.4 mmol/l (SE) in placebo to 1.8±0.7 mmol/l in Fur (P=0.04), which caused changes in relative contribution of erythrocyte Na^+ to ΔSID from 15.3±5.7% in placebo to 29.8±8.0% in Fur (P=0.03). Furosemide had no effect on $[H^+]_p$ in pulmonary circulation. This was caused by adaptations in Cl^- and Na^+ fluxes across the erythrocyte membrane.

Ability of Thoroughbred racehorse trainers to predict the use of furosemide in Thoroughbred racehorses

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Incidence and severity of exercise-induced pulmonary haemorrhage (EIPH), a common disorder of racehorses, is decreased by pre-race application of furosemide. The purpose of the study was to determine the ability of horse trainers to determine if furosemide had been administered to their horse. A questionnaire was supplied to horse trainers of 154 horses enrolled in a randomized, placebo-controlled, blinded, crossover field trial. The response rate was 71.3%. The questionnaire was designed to elucidate the ability of horse trainers to predict when a horse had been treated with furosemide as well as what factors contributed to this prediction. Trainers correctly predicted administration of furosemide, vs placebo, on 75.4% of occasions on which furosemide was administered and trainer prediction of treatment was strongly associated with the treatment given (OR=9.51). Trainer perception of the horse's performance (better, met, or below expectations) was not influenced by their opinion of whether the horse had received furosemide (P=0.072). Urination (P=0.009) and hydration (P=0.008) were significantly associated with correct treatment prediction, while performance and behaviour were not. Trainers are able to predict whether a horse has been treated with furosemide the majority, though not all of, the time. These results suggest that the effects of furosemide treatment are not always qualitatively observable by trainers.

Influence of long-term furosemide administration on bone mineral content and bone metabolism markers

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Furosemide is a diuretic used to reduce the incidence of exercise induced pulmonary haemorrhage (EIPH) in racehorses. Previous research suggests furosemide negatively influences calcium balance and may have long-term implications for bone health. In this study, furosemide use was evaluated over 56 d for effect on bone mineral content (BMC) as well as osteocalcin (OC) and pyridinoline cross-links (PYD). Mature horses were randomly assigned to either control (CON, n=5) or treatment (FUR, n=6). Once weekly, FUR horses were administered furosemide (IV) at 1 mg/kg BW. Radiographs of the left third metacarpal were taken on d -28, d 0, d 28, and d 56 for determination of cortical BMC using radiographic bone aluminium equivalence (RBAE). Venous blood samples were collected on treatment days and 24 h post administration to measure OC and PYD concentrations. Data were analysed using a mixed model ANOVA with repeated measures. No treatment effects were observed for BMC, but there was a period effect across all cortices (P<0.0001), with BMC decreasing during stalling. Osteocalcin showed no difference between groups (P=0.26) or days (P=0.25). Change from baseline in PYD tended to be greater in FUR (FUR=0.26±0.33 ng/ml; CON=-0.72±0.37 ng/ml; P=0.0584) and exhibited a day effect (P<0.0001). While there was no treatment effect observed for BMC, the trend toward greater changes in PYD in FUR may warrant further investigation with different imaging techniques or an alternative timeline.

Does oral magnesium aspartate supplementation affect reaction speed in horses of different breeds?

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Magnesium (Mg) aspartate supplementation has been shown to reduce reaction speed (RS) in six Standardbreds (total 0.06 g Mg/kg BW). To confirm and explore the potential role of magnesium or aspartate, 6 Thoroughbreds, 6 Arabians and 6 ponies were fed 3 different hay-based rations (A: control: 0.03 g Mg/kg BW; B: Control plus magnesium aspartate (same supplementation level as the Standardbreds): 0.05 g Mg/kg BW and C: Control plus sodium aspartate: 0.03 g Mg/kg BW) in a Latin square design with a 7-day washout period. Initially horses underwent a blind crossover test to score sedation and measure RS, using previously validated methods, when administered a mild anxiolytic (0.04 mg/kg BW acepromazine [ACP]) or a placebo. Each dietary treatment was then fed for 7 days with RS testing and sedation scoring (SS) on Days 1 and 7. A linear mixed model for RS and a generalised mixed model for SS were fitted, with day, breed and treatment as fixed effects and horse and group as random effects using ASReml-R. With ACP there was a reduction in RS (P=0.004) and greater sedation (P=0.001) vs placebo. There were individual differences in dietary responses but overall no significant effect on RS or SS after either one or seven days of supplementation. Potentially the total magnesium content of the ration must be ≥0.06 g Mg/kg BW, or breed compounded the previous results, or the Standardbreds, coming from the same training yard, were a homologous group. Magnesium supplementation should not be relied upon to modify reaction speed in performance horses.

Effects of furosemide, blood depletion, and exercise on body fluid compartments in Thoroughbreds

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The objective was to assess body fluid compartments under three conditions: Test 1. after supramaximal exercise; Test 2: after administration of furosemide; Test 3: after removal and re-infusion of blood. Bioimpedance spectroscopy (BIS) was used as a validated non-invasive assessment of total body water (TBW), extracellular fluid (ECF) and intracellular fluid (ICF). Six Thoroughbreds were assessed using BIS in a controlled crossover randomized trial. BIS scans were taken 1: before and after a treadmill exercise test to 120%VO_{2max} (Test 1); 2: before and 4 h after 0.5 mg/kg IV furosemide (Test 2); 3: before and 4 h after 12 l of blood was rapidly removed, then again after blood re-infusion (Test 3). Data median and interquartile ranges are shown. Differences in body composition were examined using Wilcoxon signed rank tests ($P<0.05$). Test 1: Short, high intensity exercise had no effect on fluid compartments. Pre- vs Post-exercise TBW, ECF and ICF were 337 l (318-362) vs 333 l (314-352), 110 l (105-121) vs 108 l (105-126), 225 l (220-245) vs 235 l (216-243), respectively. Test 2: Administration of furosemide decreased TBW by 27 l (22-35) from 337 l (318-362) to 310 l (296-327) ($P=0.04$) and decreased ECF by 5 l (7-9) from 110 l (105-121) to 106 l (96-113) ($P=0.03$). ICF decreased by 13 l (17-18) ($P=0.06$). Test 3: Re-infusion of previously removed blood increased TBW by 10 l (2-15) from 334 l (328-343) to 344 l (330-358) ($P=0.03$). ICF increased by 8 l (9-12) from 227 l (215-233) to 235 l (224-245) following re-infusion ($P=0.03$). There was no significant effect of blood depletion alone compared to baseline. Furosemide and iatrogenic blood volume changes induced body fluid shifts that were detected using BIS, whereas short duration maximal exercise did not induce significant changes.

Cardiovascular and Respiratory

EIPH: effects of blood volume changes on left ventricular, pulmonary arterial and venous pressures

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Pulmonary capillary pressures reflect left heart and pulmonary venous pressures (PAWedge) rather than pulmonary artery pressure (PAP). We altered circulating blood volumes to change these pressures to assess the contribution of left heart pressure and PAWedge to EIPH. Six Thoroughbreds with consistent EIPH participated in a controlled trial using a treadmill exercise test to fatigue at 120%VO_{2max}. Horses were exercised and weighed before (T-0:Control), 4 h following removal of 14 l of blood (T-18h) and 4 h after re-infusion of that blood (T-26h). Water intake was monitored. Catheters were introduced to measure left ventricular pressure (LV), PAP, and PAWedge during exercise. Endoscopic EIPH scoring (EIPHscore) and blind BAL with erythrocyte count (BALrbc) were also performed 60 min post-exercise. The parameters' averages were compared between runs using a Friedman test and associations evaluated with a Spearman rank test ($P<0.05$). Severity of EIPH shown by EIPHscore ($P=0.01$) and BALrbc ($P=0.003$) decreased, then increased significantly with blood depletion and re-infusion. Mean LV ($P=0.006$), PAP ($P=0.04$) and PAWedge ($P=0.04$) decreased, then increased with blood depletion and re-infusion, respectively. A strong correlation existed between EIPHscore and BALrbc (rs=0.63; $P=0.006$) and between PAP and PAWedge (rs=0.55; $P=0.02$). LV and PAWedge (rs=0.52; $P=0.03$) but not PAP were correlated. A moderate association existed between LV and BALrbc (rs=0.51; $P=0.03$), but not PAP or PAWedge and BALrbc (due primarily to high BALrbc in two horses) or EIPHscore. Blood depletion and repletion had an effect on LV and PAWedge and affected EIPH severity. PAWedge increases may be secondary to end-diastolic LV increases.

Respiratory abnormalities and cardiac arrhythmias during high-speed treadmill exercise in horses

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A retrospective study was performed to investigate potential relationships between respiratory abnormalities and exercise-associated cardiac arrhythmias in poorly performing horses. Horses that completed a high-speed treadmill exercise test consisting of dynamic upper airway video-endoscopy, arterial blood gas evaluation, electrocardiograms (ECG), and post-exercise echocardiograms and bronchoalveolar lavages (BAL), were included. Fifty-seven horses met the criteria, of which four were healthy controls (58% Thoroughbreds, 38% Standardbreds and 4% Quarter-horses; 31 males and 26 females; mean age=4.5 years). Seven (13%) had dynamic upper airway obstruction (DAO) only, 30 (57%) had lower airway inflammation (IAD) only and 11 (21%) had both DAO and IAD; five (9%) had only cardiac abnormalities. Arrhythmias were identified in 41 (72%) horses during or after maximal exercise (26 (46%) had simple isolated premature depolarisations and 15 (26%) had complex ectopic or re-entrant rhythms). The presence of lower airway abnormalities was significantly associated with simple and complex arrhythmias in any phase (pre, during and post exercise) (OR 5.30). Evidence of exercise-induced pulmonary haemorrhage (EIPH) on BAL was significantly associated with arrhythmias in any phase (OR 6.33) and with presence of IAD (OR 8.18). Diagnosis of left ventricular hypokinesis on post-exercise echocardiography was significantly associated with complex arrhythmias during and after exercise (OR 26.4). No significant associations were found between cardiac arrhythmias and breed, age, DAO or blood gas measurements. Concurrent cardiorespiratory abnormalities were a common finding, highlighting the importance of multimodal diagnostics when investigating poor performance. The association between lower airway disease and cardiac arrhythmias warrants further investigation.

Endoscopic evaluation of the upper airway at rest and during maximal treadmill exercise in 75 horses

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A retrospective study was performed to investigate potential relationships between upper respiratory abnormalities at rest and during maximal-speed treadmill exercise in a mixed population of horses referred for poor athletic performance (2011-2017). Horses that underwent video-endoscopy at rest and during maximal-speed treadmill exercise were selected. Laryngeal function was graded using the Havemeyer system at rest and the A to C system during exercise. Resting and dynamic endoscopic findings were correlated. 75 horses met the criteria (59% Thoroughbreds, 35% Standardbred and 7% other; mean age=4.7 years old; 32 females and 43 males). Dynamic airway obstruction (DAO) was diagnosed in 29 (39%) horses; the most common findings were pharyngeal collapse (PC) (13%), left arytenoid collapse (12%) and dorsal displacement of the soft palate (8%). 97% of horses had grade 1 or 2 resting laryngeal function and 12% had moderate or severe pharyngeal lymphoid hyperplasia. Resting laryngeal function was not correlated with laryngeal function at maximal exercise (r=0.35). 89% of horses with significant dynamic arytenoid collapse had grade 1 or 2 resting laryngeal function. Moderate or severe pharyngeal lymphoid hyperplasia was significantly associated with PC (OR 20.25, $P<0.001$). No significant associations were found between presence or type of DAO and breed, age, sex or arterial blood gas measurements. Findings indicate that resting laryngeal function is not a good predictor of upper respiratory function at maximal exercise and highlight the need for dynamic video-endoscopy to reliably diagnose causes of poor performance. The association between pharyngeal inflammation and dynamic PC warrants further investigation.

Effect of exercise-induced pulmonary haemorrhage on career longevity and performance in Thoroughbred racehorses

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Exercise induced pulmonary haemorrhage (EIPH) has a high incidence among Thoroughbred racehorses, but only one study has investigated long-term impacts of EIPH on career performance. We sought to determine if EIPH is associated with career performance (longevity, races, wins, places, and earnings) among a population of South African Thoroughbred racehorses not treated with furosemide or nasal dilator strips, in a pooled prospective, longitudinal study. A total of 1,034 horses underwent a single post-race tracheobronchoscopic examination, with EIPH independently evaluated as severity grade 0-4 by three investigators blinded to the horses' identity and race performance. Racing records for all horses were obtained from a commercial database after retirement from competitive racing and were summarized into career performance variables. Overall, 70% of horses showed evidence of EIPH. Controlling for age, sex, and location, horses with EIPH started in approximately four more races than horses without (95% CI 1.99 to 6.18). Lifetime earnings were significantly, but not meaningfully, higher among horses with EIPH grade >1 compared with those with EIPH grade 0 (adjusted average change in earnings 1.69 Rand, 95% CI 1.08 to 2.64). EIPH was not significantly associated with career longevity (adjusted Hazard Ratio 0.88, 95% CI 0.77 to 1.01), lifetime wins, or lifetime places. EIPH does not appear to be associated with career performance.

Cardiac dimensions and function in matched untrained vs endurance trained Arabian horses

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The aim of this study was to compare cardiac dimensions and function between high-level (>100 km) endurance-trained and matched untrained Arabian horses from similar bloodlines by standard echocardiography and 2D speckle tracking (2DST). Echocardiography was performed in 13 untrained (11±3 years, 448±24 kg) and 13 high-level endurance-trained (11±3 years, 434±53 kg) Arabian horses (without more than mild valvular regurgitation; >2 weeks after strenuous exercise). Systolic and diastolic left ventricular (LV), left atrial and aortic dimensions and LV volume were determined. From long and short axis 2DST images, strain rate and velocity were determined in several myocardial wall segments. Groups were compared by an independent samples t-test ($P<0.05$). In trained horses systolic and diastolic LV diameter (6.6±0.9 and 10.9±1.0 cm), area (68±10 and 141±18 cm^2) and volume (332±85 and 1,097±200 cm^3) were significantly higher than in untrained horses (5.8±0.8 cm, 10.2±0.6 cm; 56±6 cm^2, 127±11 cm^2; 258±47 cm^3, 866±86 cm^3, respectively). LV fractional shortening and fractional area change, aortic and left atrial dimensions, were not different between groups. From 2DST images, a significantly lower longitudinal velocity (7.83±1.31 m/s), longitudinal (0.63±0.16 s^{-1}), circumferential (0.54±0.12 s^{-1}) and radial (1.32±0.23 s^{-1}) strain rate during atrial contraction was seen in trained horses compared to untrained horses (9.44±1.19 m/s, 0.83±0.13 s^{-1}, 0.68±0.08 s^{-1}, 1.70±0.54 s^{-1}, respectively). In conclusion, this study investigated the cardiac effects of endurance training in adult age- and bodyweight matched Arabian horses. Endurance training induces LV enlargement. In trained horses, atrial contraction induces less pronounced movement of the LV wall. Some pathological conditions induce similar alterations, so the level of training should be taken into account when evaluating cardiac function in sport horses.

Prevalence and repeatability of exercise-induced arrhythmias in Standardbred racehorses in training

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The objective was to investigate the prevalence of arrhythmic events and repeatability of electrocardiogram (ECG) findings in Standardbred (SB) racehorses in training. A convenience sample of SB racehorses was examined during routine fast work. Continuous ECG recordings and GPS data were recorded simultaneously. Additional recordings were made to investigate the repeatability of ECG findings in a subset of horses. Premature depolarisations (PD) were identified as deviations in the R-R interval of >20% at rest and >8% during and immediately after exercise. Arrhythmias were classified as simple (isolated PDs) or complex (couplets or more) and tachyarrhythmias were defined as runs of >3 consecutive PDs. Data were analysed from 36 horses with complete ECG recordings (23 geldings, 13 mares; age: 2-9 years). Median distance and peak speed during strenuous exercise were 2,250 m (range: 2,000-6,000 m) and 14.3 m/s (range: 12.5-15.2 m/s), respectively. Median peak heart rate was 235 bpm (range: 197-250 bpm). Six (17%) horses had isolated PDs at rest and 36 (83%) horses had one or more PDs identified during (n=29) and/or after (n=25) strenuous exercise, on the first examination. Twenty-one horses (58%) had complex arrhythmias, including 9 (25%) that had episodes of tachyarrhythmia. No significant associations were found with age, speed, distance, fitness or performance level. Additional ECG recordings with useable traces were available for 12 horses. Differences in arrhythmia classification were observed in 9/12 (75%) horses. Exercise-induced arrhythmias were a common finding in apparently healthy strenuously exercising SB racehorses in training. The classification of arrhythmias was not consistent on subsequent exercise tests.

Evaluation of cardiac arrhythmias in horses performing below expectation in Thoroughbred races in Hong Kong

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Arrhythmias may cause poor performance but the frequency and outcome of this diagnosis is not well described. Veterinary records of Thoroughbred horses in Hong Kong undergoing post-race veterinary examination for performing below expectation from 2007-2017 were reviewed. In 97,738 starts arrhythmia was diagnosed in 269 episodes (0.0027% of starts) in 237 horses. Atrial fibrillation (AF) was confirmed in 39 episodes and frequent VPC in one episode. In 229 episodes the arrhythmia was not further characterised. AF persisted beyond 72 hours in 11 horses. Sinus rhythm was achieved after treatment in 9/9 horses. In horses with only one episode of arrhythmia, 54/198 were retired immediately. In the remaining horses with only one episode of arrhythmia, mean career duration was 626 days, range 46-2,352. One horse diagnosed with post-race arrhythmia died of sudden death 735 days later. Thirty -eight horses had two episodes of arrhythmia. In these horses, the mean duration between episodes was 400 days, range 35-1,066. Twenty-eight of these horses were retired immediately after the second episode. One horse had three episodes of arrhythmia and was immediately retired after the third episode. One-hundred and twenty-six horses won at least one race after the arrhythmia. In conclusion, cardiac arrhythmias are infrequently diagnosed on post-race examinations and the majority of episodes are paroxysmal. Horses can perform well following and between episodes. Horses diagnosed with arrhythmias are at increased risk of recurrence, although this may not occur for many years. The relationship between paroxysmal or persistent post-race arrhythmia and sudden death is unclear.

Exercise-induced pulmonary haemorrhage in show-jumping horses

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The aim of this prospective clinical study was to evaluate the prevalence of exercise-induced pulmonary haemorrhage (EIPH) in a population of 922 competitive showjumping horses. Horses were referred to a specialized sports medicine practice for routine evaluation or a complaint related to performance. A respiratory endoscopy, a tracheal wash (TW) and a bronchoalveolar lavage (BAL) were performed on all horses. The diagnosis of EIPH was established when haemosiderophages represented more than 5% of leucocytes in the TW and/or BAL. The prevalence of EIPH was 22.8% in the overall population. Only 9% of the EIPH cases presented with post-exercise epistaxis. EIPH was associated to perceived breathlessness or increased recovery time after competition. Owners complained of a true loss of performance in only 10% of cases. Repeated EIPH and competing at higher level increased the risk of having reduced performances. Inflammatory airway disease was diagnosed in 77.8% of all horses and in 90.5% of the EIPH cases. A highly significant association was found between EIPH and IAD (OR=7.0, CI 4.3-11.3; $P<0.0001$). Horses with primary or secondary respiratory bacterial or fungal infection were not at a higher risk of having EIPH, however identification of fungal particles in any sample increased the risk of EIPH (OR=2.2, CI 1.6-3.2; $P<0.0001$). It was concluded that EIPH is markedly prevalent in showjumpers, especially those competing at high-level. Because of their longer careers, timely diagnosis of EIPH is warranted in these athletes to avoid loss of performance. The treatment of associated lower airway inflammation is important to address.

Comparison of VO_{2max} and measures of ventilation during racetrack and treadmill exercise

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The extent to which measurements of VO_{2max} and ventilation made during treadmill exercise (TM) relate to those made during maximal racetrack exercise (RT) has been pondered by many investigators but remains unclear, so measurements of these variables were compared in the same group of horses exercising maximally on each surface. Six fit horses completed a controlled, randomized, crossover study in which they galloped 1,600 m at top speed under saddle on a racetrack, and ran to fatigue on a treadmill (6% slope) at 115%VO_{2max}. Breathing frequency (f_b), inspiratory and expiratory volumes (V_I, V_E), minute ventilation (V_{min}), peak inspiratory and expiratory flow rates (pkV_I, pkV_E) and VO_{2max} were measured using an ergospirometer worn throughout each exercise bout. For TM, horses ran wearing a saddle plus weighted saddle bags equal in total weight to those of the rider plus saddle. These weights were included in mass-specific VO_{2max} calculations. Results were analysed by RM ANOVA and post hoc tests, with significance set at $P<0.05$. VO_{2max} did not differ ($P=0.13$) between TM (149±12 ml/kg/min) and RT (155±12 ml/kg/min). There was no difference in f_b (RT:138±8.8/min; TM:130±3.6/min; $P=0.09$). No differences were detected in V_I (RT:14.5±1.2 l; TM:14.7±0.9 l; $P=0.74$), pkV_I (RT: 81.8±7.2 l/sec; TM:80.3±3.5 l/sec; $P=0.51$) or V_{min} (RT:1,930±163 l/min; TM:1,991±125 l/min; $P=0.25$). V_E (RT:13.8±1.4 l; TM:15.5±1.5 l; $P=0.01$)and pkV_E (RT:84±9.7 l/s;TM:97±10.9 l/s; $P=0.006$) were both lower with RT. There were no differences between VO_{2max}, f_b, V_{min}, V_I or pkV_I on the racetrack or treadmill. The lower RT V_E and pkV_E were unexpected. Impelling of air into the ergospirometer throughout RT galloping might artificially reduce expiration-related measurements.

Variation in laryngeal function of Thoroughbred racehorses seen at exercise on two occasions

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Grading of laryngeal function at exercise now largely forms the basis for surgical decision-making for recurrent laryngeal neuropathy. This assessment is complicated by intra-observer, inter-observer and intra-horse variation. Although previously reported at rest, significant intra-horse variation has not been documented at exercise. The objective of this study was to determine the degree of variation in arytenoid function at exercise when Thoroughbred racehorses were assessed on two separate occasions in a prospective cohort study. 48 racehorses in training underwent overground endoscopy on two separate occasions no more than 60 days apart. Blinded and randomised recordings of each examination were analysed by the senior author and graded at rest and exercise using objective measurement based on left:right ratios. A mixed effect model and Spearman rank correlation were used in analysis of data. Considerable variation was seen between the first and second assessments at exercise. Based on the Rakestraw three point grading system, 7/48 (14.6%) were found to have improved in exercising grade and 6/48 (12.5%) to have deteriorated in grade. As a population however, the group was found to neither improve nor deteriorate overall. Weak correlation was identified between the variation in exercise grades and that of resting grades. Further examinations would have allowed evaluation of continued variation with time, however, this study demonstrates a considerable variation in laryngeal grade over periods as short as 60 days. This highlights the need for judicious interpretation of overground endoscopy with possible requirement for repeat examinations prior to important treatment decisions.

Medical treatment of dorsal displacement of the soft palate in sport horses

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Dorsal displacement of the soft palate (DDSP) is well described in race horses and is typically treated surgically. The aim of this retrospective study was to describe the condition in sport horses and evaluate the success of medical treatment. Records from 270 overground endoscopies performed on sport horses over a period of 3 years were reviewed. Twenty-seven horses had DDSP during exercise. At the time the DDSP occurred, it was associated with a cough (22/27 horses), another noise (4/27 horses) and was silent in one horse. Tracheal inflammation was present in 25/27 cases. A bronchoalveolar lavage was performed in 24 cases and all had evidence of lower airway inflammation. Culture of the tracheal wash was positive for bacteria in 14/27 horses and for fungi in 16/27 horses. Medical treatment was administered to 25 horses. This involved topical anti-inflammatory treatment with furacine and DMSO (n=16) and/or inhaled corticosteroids (n=25), bronchodilators and antimicrobial (n=17) therapy. Follow-up included a recheck overground endoscopy in three horses, recheck of the respiratory samples in eight horses and interview of 23 owners/riders. Treatment was successful in 22/25 cases. These horses stopped coughing or making a noise and were performing up to expectation. Treatment failures occurred in two very chronic cases and one case where the DDSP occurred after tie-back surgery. Limitations of the study include a lack of control groups and a large reliance on owner feedback as a measure of improvement. In this sport horse population, DDSP is associated with lower airway inflammation in a large proportion of cases and can be managed medically.

Plasma amino acid concentrations during treadmill exercise tests in horses with cardiac disease

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Ion channelopathies can cause cardiac arrhythmias by destabilising cellular membrane potentials. N-methyl D-aspartate receptors (NMDAR) in cardiomyocyte cell membranes act as cation channels. Amino acids (AA) like glutamate or glycine are agonists of NMDARs. Equine plasma concentrations of AAs ($[AA]_p$) during exercise are poorly described. Our aim was to measure $[AA]_p$ in healthy horses during a sub-maximal high-speed treadmill standardised exercise test (HSET) and to compare to horses with cardiac disease. Eleven healthy (group H) and five horses with cardiac disease (group C) were evaluated using a HSET. Blood samples were taken at rest, during and after exercise. Lactate, $[AA]_p$, velocity, heart rate (HR) and rhythm were measured. Performance, lactate and $[AA]_p$ between healthy and cardiac diseased horses were compared using Student t-tests and 2-way repeated measures ANOVA ($P<0.05$). Performance indices were not different between groups (V_{150}, V_{200}, peak velocity, HR, lactate). Elevated lactate was seen in Group C horses during recovery. Three horses in Group C had arrhythmias during the HSET while one in Group H had an arrhythmia. Twenty-four AAs were measured. Each $[AA]_p$ increased from baseline during and immediately after exercise. No differences between Group C and H horses were detected for any AA at any time point. Alanine showed the largest increase with exercise. Samples should be taken during and immediately following exercise to capture the magnitude of this $[AA]_p$ increase. Lactate clearance should be further investigated, as it may be useful to distinguish between cardiac and other causes of poor performance.

Evaluation of late potentials for prevention of sudden death in Thoroughbred horses

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Sudden cardiac death has sometimes occurred in horses. Because the ventricular late potentials (LP) detected on the body surface by signal-averaged electrocardiography (SA ECG) are thought to arise from injured tissue generating prolonged ventricular activation, LP are considered as a portent marker of sustained ventricular tachycardia in humans. Because this analysis in horses did not validate, reference values are essentially missing. The aim of this study was to create reference values for SA ECG in healthy horses. Twenty-seven healthy Thoroughbred horses (age 3-32 years old) were investigated. After careful skin preparation, electrodes were attached, and high-resolution ECGs were recorded from the X, Y, and Z leads by Holter ECG recorder at rest in the stall. Horizontal lead (X) was placed in the 4th intercostal space on the left and right side. The vertical lead (Y) was placed on the proximal part of the sternum and above the sacral bone. Sagittal lead (Z) was placed in 4th intercostal space parasternally and on the corresponding position paravertebrally on the left. Filtered QRS duration (fQRS), duration of low amplitude signal below 40μV (LAS40) and root mean square voltage of the last 40 milliseconds (ms) of the QRS (RMS40) were measured. The values of the SA ECG parameters were tested with the Kolmogorov-Smirnov goodness-of-fit test for normality. Although the fQRS (127.7±19.5; mean±SD) showed a normal distribution, the distribution of the LAS40 (96.5±38.5) and RMS40 (152.0±72.5) was biphasic. Older animals showed a tendency to have longer LAS40 and smaller RMS40. Only a few old horse LP developed. From these results, normal values are fQRS<150 ms; LAS40<100 ms; and RMS40>80μV. Presented results could offer a basis for further arrythmology studies in horses.

Assessment of left ventricular function in horses with aortic regurgitation by 2D speckle tracking

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The aim of this study was to examine if 2D speckle tracking (2DST) could detect changes in left ventricular (LV) myocardial wall motion in horses with different grades of chronic aortic regurgitation (AR) compared to control horses. Standard echocardiography and 2DST were performed in 29 healthy Warmblood horses (8±4 years, 566±48 kg) and 57 Warmblood horses with AR (15±6 years, 548±68 kg), divided in subgroups with mild (n=23), moderate (n=21) or severe AR (n=13). Speckle tracking was performed on LV short axis (6 segments) and long axis images (4 segments). Myocardial wall motion was evaluated by strain, strain rate (Sr) and displacement for all segments separately. Horses with moderate AR showed significantly (P<0.05) higher radial (75.5±24.3%) and circumferential strain (19.3±3.2%) compared to control horses (54.5±18.0%, 16.8±3.0%, respectively). In the interventricular septum, longitudinal displacement was positively correlated with increasing AR severity. Radial, circumferential and longitudinal early diastolic Sr was lower in several segments in horses with AR, whereas late diastolic Sr was higher. This may be influenced by the difference in age between control horses and horses with AR. In conclusion, AR can lead to LV dilatation and arrhythmias, which may increase the risk for sudden cardiac death. A good follow-up of the progression of AR severity is paramount in sport horses. 2DST is able to detect altered myocardial motion in horses with AR. Further research is needed to distinguish the effects of age from the effects of AR on myocardial wall motion and determine the clinical usefulness of these findings.

Coughing and dysphagia induced by prosthetic laryngoplasty – outcome following prosthesis removal

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Prosthetic laryngoplasty (LP) is the mainstay of treatment for horses with recurrent laryngeal neuropathy. It is recognised that LP carries a high rate of post-operative complications, such as coughing and dysphagia (C&D), which may ultimately necessitate prosthesis removal. There are no published studies reporting the outcomes for horses that undergo laryngoplasty prosthesis removal (LPR). A telephone or paper questionnaire follow up was undertaken for all horses that underwent prosthesis removal between September 2003 and June 2017. Fifty-two horses underwent LPR due to signs of C&D. Adequate follow up information was available in 32 horses. The population included 11 Thoroughbred racehorses, 6 Thoroughbred/cross eventers, 4 Warmblood sport horses, 8 hunters and 3 others. The degree of arytenoid abduction before LPR was graded using the Kidd & Slone scale out of 5, whereby the greater the number, the greater the abduction; grade 1: 3 horses, grade 2: 3 horses, grade 3: 24 horses, grade 4: 2 horses, grade 5: 0 horses. C&D were not associated with the degree of abduction and may be associated with the presence of the prosthesis. Following LPR, 21 horses (66%) showed a substantial improvement with the presenting clinical signs no longer exhibited, and a further 3 horses showed some improvement. Twenty-three horses (72%) returned to full athletic function. In 42% of horses, LPR resulted in an increase in respiratory noise during exercise compared to with the prosthesis *in situ*. LPR is a worthwhile option in the management of horses experiencing intractable coughing and dysphagia following LP.

Effects of Omeprazole on electrolyte balance and exercise arrhythmias

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Omeprazole use in humans has been associated with an increased risk of cardiac arrhythmias, which has been potentially attributed to hypomagnesaemia. The aim of this study was to investigate the effects of omeprazole treatment on electrolyte balance and arrhythmia prevalence in strenuously exercising racehorses. A convenience sample of 20 Standardbred horses, in race training, was examined (10 treatment, 10 control). Blood and urine were collected before exercise for measurement of serum electrolyte concentrations and urinary fractional excretion (FE). Horses completed a standardised exercise test on the track, including 2,000 m warm up, 2,000 m fast work and 2,000 m recovery, during which ECGs were recorded. Of the treated horses, eight received a dose of 4 mg/kg omeprazole and two received 2 mg/kg. Median duration of treatment was 7 weeks (range: 5 weeks to >2 years). Arrhythmias were identified in 15/20 (75%) horses during (n=14) and after (n=12) strenuous exercise. Six had isolated premature depolarisations and nine had complex rhythms (couplets or more). No significant difference in arrhythmia prevalence during and after strenuous exercise was found between groups (omeprazole 90% vs control 60%). Serum total calcium concentration (mean±SD) was lower in controls vs treated horses (2.86±0.09 vs 2.98± 0.10 mmol/l; P=0.01), although both were within the reference interval. No other differences were found in serum concentrations or FE of electrolytes. In conclusion, no evidence was found of an increased risk of electrolyte imbalance or arrhythmia prevalence in association with omeprazole usage in this small study.

Comparison of EIPH severity in horses engaged in high intensity racetrack and treadmill exercise

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Multiple treadmill-based studies using low numbers of horses have evaluated potential treatments for exercise-induced pulmonary haemorrhage (EIPH) and found no effect. However, the relevance of these findings to racing is unclear. Because severity of EIPH incurred on treadmills has not been compared to that following high-speed racetrack exercise in the same horses, we retrospectively performed this comparison using bronchoalveolar lavage (BAL) due to the relative insensitivity of tracheobronchoscopy. Six race-fit Thoroughbreds with recent tracheobronchoscopic EIPH scores ≥2 were exercised to fatigue on a treadmill at 115%VO_{2max} (5% incline, 12.3-14.2 m/s), and maximally on a racetrack over 800 m and 1,100 m with average speeds ranging from 16.4-16.7 and 15.5-16.6 m/s, respectively. Run order varied but was not randomized. BAL was performed blindly using Bivona tubes 45-60 mins post-exercise. BAL erythrocyte numbers (BALRBC) were determined using a haemocytometer. Data were analysed using RM ANOVA and expressed as mean±SD (P<0.05). BALRBC were greater after racetrack exercise than after treadmill exercise (treadmill: 16,899±20,640/µl [range: 3,275-58,000/µl]; 800 m: 41,640±30,330 [12,700-82,500/µl]; 1,100 m: 274,467±610,857 [5,850-1,520,000/µl]; P=0.049). Treadmill exercise resulted in lower numbers and a narrower range in BALRBC than racetrack exercise. Thus, when a small number of horses is used to study EIPH treatments on a treadmill, a lower control BALRBC would be anticipated than with a similar study using racetrack exercise, and might reduce the likelihood of demonstrating significant treatment effects. Results of this retrospective study raise concern regarding the advisability of extrapolating conclusions regarding efficacy of EIPH treatments from treadmill studies to racetrack scenarios.

Effects of large changes in blood volume on VO_{2max}

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Exercise-induced splenic contraction in horses expands the circulating blood volume and raises the oxygen carrying capacity of the blood. However, the effects of large changes in blood volume on VO_2, particularly VO_{2max}, have not been directly measured. This study quantitated the effects of changes in blood volume on VO_{2max} in supramaximally exercising Thoroughbreds. Six fit Thoroughbred racehorses exercised to fatigue on a treadmill at 120%VO_{2max} in a controlled trial: T0h=baseline, 4 h following removal of 14 l of blood (T18h=depletion run) and 4 h after re-infusion of that blood (T26h=repletion run). Horses had free choice hay and water from T0-T18 and drank 4 l after the T18 run. VO_2 was measured throughout exercise with an ergospirometry mask. Maximum heart rate (HR_{max}) and each pre-exercise body weight were recorded. Results were analysed by RM ANOVA and post hoc tests (P<0.05). Depletion and re-infusion of blood had a significant effect on body weight (T0h=491±37 kg; T18h=480±35 kg; T26h=497±32 kg; P<0.001). HR_{max} did not change. Alterations in blood volume had a significant effect on VO_{2max} (P<0.001). Re-infusion VO_{2max} (185±20 ml/kg/min) was greater than baseline (167±16 ml/kg/min; P=0.015) by 11%, even though only the removed volume was re-infused. Baseline and depletion (155±16 ml/kg/min) VO_{2max} differed by 7% (P=0.055). Large changes in blood volume can have a profound effect on VO_{2max}. Depletion of large volumes may have been partially offset by absorption of fluid from the gastrointestinal tract. This, plus ingestion of 4 l water prior to re-infusion, may have created a hypervolemic state which improved perfusion and oxygen delivery to muscles, and increased VO_{2max}.

Left cardiac pressures at rest and during exercise

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It has been postulated that EIPH is a consequence of pulmonary capillary stress failure arising from high end-diastolic left atrial pressures (PLA_{ED}) caused by volume overload secondary to splenic contraction. The anatomic relationship of the aortic trunk to the LA has prevented direct LA pressure measurement during exercise despite major technological advances. Therefore, confirmation that LA volume overload causes pulmonary capillary stress failure is lacking. However, as classical cardiac cycle pressure measurements demonstrate, there is a relationship between PLA_{ED} and diastolic LV pressure (PLV_D) which could enable accurate estimations of PLA_{ED} from the LV_D. Before undergoing an incremental treadmill exercise test, a retired Thoroughbred racehorse (EIPH score=2) with normal heart valves had a catheter placed in the LV via an exteriorized carotid loop. Using a steerable guide which turned 180° and ultrasound, another catheter was passed via the other carotid into the LA lumen. Both catheters were identical and had solid-state pressure sensors at their tips. At rest with HR=54, mean±SD $PLA_{Systolic}$=79±1.9 mmHg and PLA_{ED}=15±2.6 mmHg, respectively. PLV_D equalled PLA_{ED} 170±28 msec prior to LV_{ED}. During exercise with HR=190, $PLA_{Systolic}$=120±21 and PLA_{ED}=60±5 mmHg, while $PLV_{Systolic}$=240±11.5 and PLV_{ED}=127±21.1 mmHg. For HR≥186 bpm, PLV_D equalled PLA_{ED} 70±4 (range: 62-76) msec before LV_{ED} and did not rise until $LA_{Systole}$ began. PLA exceeded the estimated stress failure threshold of pulmonary capillaries (~75 mmHg) but was technically extremely difficult to measure. Approximating PLA_{ED} from PLV_D 60-75 msec before LV_{ED} may be possible, would obviate the need to directly measure PLA_{ED}, and might facilitate investigations of the relationship between PLA_{ED}, blood volume and EIPH.

Identification of N-methyl D-aspartate receptor subunits in equine brain and myocardium using PCR

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Ion channelopathies can cause cardiac arrhythmias by destabilising cellular membrane potentials. N-methyl D-aspartate receptors (NMDAR) were recently identified in cardiomyocyte cell membranes and are thought to act as cation channels. NMDAR agonism has been associated with arrhythmia development. Multiple subtypes of NMDARs are recognised, differing in their subunit composition (7 known subunits) and biophysical and pharmacological properties. Our aim was to characterise and compare the NMDAR subunit expression in equine myocardium and brain tissue samples. Tissue samples (right and left atria, right and left ventricle, cortex, cerebellum, brainstem) from five horses euthanised for non-cardiac disease were obtained immediately after death and stored at -80 °C until processing. Primers were designed for the known equine NMDAR subunit sequences (GRIN1, 2A, 2B, 2C, 2D, 3A, 3B). mRNA was extracted from the stored tissues and reverse transcribed into cDNA for subsequent PCR reaction. Both qualitative and quantitative PCR were performed. Sanger sequencing of PCR products confirmed amplification of the target sequences. NMDAR subunits GRIN1, 2A, 2B and 2D were identified in both myocardial and brain samples, while GRIN 2C (cerebellum) and 3A (cortex) were identified in the brain only. Further research is underway to quantify regional differences in gene expression and confirm protein expression occurs. NMDARs may provide a new target for anti-arrhythmic pharmacological investigation.

Within-breath input impedance by impulse oscillometry in severely asthmatic horses

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Impulse oscillometry system (IOS) detects pulmonary obstruction in horses measuring average total respiratory system impedance (Zrs). In humans, analysis of within-breath changes of Zrs allows identification of the presence of expiratory flow limitation. Aims of this study were to describe the inspiratory and expiratory Zrs parameters measured by IOS in asthmatic horses and to determine whether they allow the detection of residual airway obstruction in asthmatic horses in disease remission. Seven severely asthmatic horses in disease exacerbation (HE), seven asthmatic horses in clinical remission (HR) and seven control horses (HC) from a cohort of experimental age-matched animals underwent IOS testing. Only data at 3, 5 and 7 Hz with coherence >0.85 at 3 Hz and >0.9 at 5 and 7 Hz were considered in this study. Mean, inspiratory and expiratory resistance (R) and reactance (X) and the difference between inspiratory and expiratory X (ΔX) were calculated at each frequency. The three groups were compared using Kruskal-Wallis and Dunn's multiple comparison tests. HE differed from HC for all R parameters at 3 Hz and all X parameters at all frequencies (P<0.01-0.001). HE differed from HR (P<0.05) for R3, R3e, X3e, X5e. HR differed from HC for X7i (P<0.05). ΔX was higher (P<0.05) in HE than in HC (5 Hz) or HR (all frequencies, P<0.01-0.001). Results indicate that as reported in humans during tidal expiratory flow limitation, Xrs during the expiratory phase is more negative than during inspiration in HE. Difference in X7i appears to be promising to discriminate between HC and HR.

Comparison of bronchoalveolar lavage fluid cytology from both lungs in Thoroughbreds

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The aims of this study were (1) to compare bronchoalveolar lavage (BAL) fluid cytological findings collected from both lungs in Thoroughbred racehorses, and (2) to determine sensitivity of the BAL procedure in diagnosing inflammatory airway disease (IAD) or exercise-induced pulmonary haemorrhage (EIPH). BAL data from both lungs were compiled from medical records of 186 Thoroughbred racehorses examined over a three-year period. A standard BAL technique, infusing 240 ml saline into each lung and pooling retrieved samples, was used throughout. Linear mixed effects models were used for analysis of cell percentages between right and left lungs. Two sets of reference values [(1) neutrophils >5% ± mast cells >2% ± eosinophils ≥1%; and (2) neutrophils >10% ± mast cells >5% ± eosinophils >5%] were used to diagnose horses with IAD. A diagnosis of EIPH was made in horses with a haemosiderophage/macrophage ratio >17. A significant difference was found between lungs for neutrophil (P=0.01), mast cell (P=0.03), lymphocyte (P=0.04) and haemosiderophage (P=0.006) percentages. Increasing age was associated with decreasing mast cell percentages. Lowest sensitivity (68%) was recorded for the left lung, when diagnosing IAD using the more conservative reference values. Left and right lung performed similarly in diagnosing EIPH, however sensitivity was low (73%). In the Thoroughbred racehorse, BALF cell distribution between lungs is not uniform. BALF should be harvested from both lungs to decrease the possibility of misdiagnosing horses free from these potentially performance limiting diseases.

Effect of tracheal collapse in tracheal inflammation and exercise tolerance in a group ponies

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There are a lack of studies that describe the clinical significance of tracheal collapse, which affects mainly small pony breeds. The aim of the study was to investigate if tracheal collapse causes inflammation in tracheal mucosa and/or exercise tolerance in affected ponies. Twenty-three healthy Shetland ponies (controls) and seven Shetland ponies with tracheal collapse were included in the study. All of the ponies had an endoscopic examination and tracheal aspirates were obtained for cytological examination. Ten controls and four ponies with tracheal collapse performed a low intensity exercise test. One year later four controls and four ponies with tracheal collapse had a follow up endoscopic examination and exercise test. The cytology of the tracheal aspirates showed no difference between the two groups. All of the ponies with tracheal collapse showed some respiratory signs during the test but there were no differences between the groups in respiratory rate after the test or heart rate during or after the test. In the follow up study two healthy controls had developed tracheal collapse and in two affected ponies the tracheal collapse had advanced. The results of this study suggest that tracheal collapse in ponies is not correlated with tracheal inflammation measured by tracheal aspiration and that ponies with tracheal collapse managed to perform an exercise test but were showing respiratory signs during the test. Tracheal collapse is a progressive disease and this study suggests that a progression can occur in as short time as one year.

Clinical alterations in horses with increased bronchoalveolar lavage fluid mast cell counts

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There are few studies of the clinical picture and significance of increased numbers of mast cells in bronchoalveolar lavage fluid (BALF) in horses. The aim of this study was to document history, clinical and laboratory findings and outcome of horses with increased BALF mast cell counts. BALF samples with increased mast cell counts (≥5%) from horses admitted to the University Animal Hospital (2015-2017), and corresponding medical records were reviewed retrospectively. In total 28 horses (mean age 9 years) had high numbers of mast cells in BALF (mean 7.1%). Breed disposition was similar to the clinic's referral population, with sport horses overrepresented (54%). The majority of horses had been referred due to exercise intolerance rather than clear respiratory signs (72%). Other clinical complaints were cough (53%) and increased respiratory pattern during exercise (46%). Almost all horses had signs of low-grade tracheal inflammation (endoscopic mucus scoring) and several horses had slightly increased eosinophil counts and 36% had evidence of EIPH in BALF. A history of seasonal problems (spring/summer) was recorded from 36% of horses; four had insect bite hypersensitivity and two simultaneous urticaria. All tested horses with seasonal symptoms were positive on equine intradermal test. The response to medical treatment was generally poor. In conclusion, while the inciting cause remains speculative, there appears to be several different aetiologies for this subtype of equine asthma and both clinical signs and response to treatment differ from neutrophilic airway inflammation.

Cardiac variables of eventing horses at rest and after cross-country in international competitions

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Cardiac disease is a known cause of poor performance and, in severe cases, of sudden death in horses. The aim of this study was to determine the prevalence of abnormal auscultation and the values of heart rate (HR) at rest and after competition. A total of 407 eventing horses, competing in CCI or CIC from 1* to 3* level in six different locations were involved in this study. To assess HR and heart rhythm, cardiac auscultation was performed at rest and respectively 5 (HR5) and 10 min (HR10) after cross-country. In addition, an electrocardiogram was recorded at rest and after exercise (AliveCor®). Statistical analyses were performed using Wilcoxon test and ANOVA with Tukey-Cramer's post-hoc test ($P<0.05$). At rest, 3.9% horses had a heart murmur, grade above 2/6, with either holosystolic (2% right and 1.2% left sided) or holodiastolic murmur (0.7%). In 81.2% of the cases, these murmurs were unknown by the rider. Also, 16.8% horses showed 2AVB, 1.2% SA and 0.2% SVES. Overall mean HR(±SD) was respectively 33(±4.6) bpm at rest, 101.3(±14.2) for HR5 and 83(±12) for HR10. Considering both format and level, there was a significant difference for HR10 between CCI* (83.3±12.1) and CCI*** (87.9±12.1), CCI** (81.3±10.8) and CCI*** and CCI*** and CIC*** (77.8±13.0). As significant valvular regurgitation could lead to arrhythmias during exercise; detection and investigation of heart murmurs is of major importance to define the potential risk for the horse. Also, reference HR values post-exercise according to level and format may help veterinarians to assess horses' recovery.

Biochemistry, Haematology, Endocrinology and Thermoregulation in Exercise

Field trial establishing the bioavailability of MicroActive CoQ10 targeting equine skeletal muscle

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Coenzyme Q10 (CoQ10, ubiquinol) plays a central role in aerobic ATP production and is a natural anti-oxidant. CoQ10 concentrations (inferred from combined electron transport chain complex activity, CI+III) are significantly lower in *myostatin* gene T:T than C:C equine skeletal muscle. This is likely due to decreased endogenous CoQ10 synthesis and can be restored *in vitro* by supplementing the assay with CoQ10. Oral CoQ10 is generally poorly absorbed although numerous commercial forms of oral CoQ10 claim enhanced bioavailability. We tested the hypothesis that oral supplementation of Microactive® CoQ10 complexed with beta-cyclodextrin would result in increased skeletal muscle mitochondrial CoQ10 in horses. Healthy, untrained Thoroughbreds (n=8 females, n=11 males, 1-3 years old) maintained on pasture on one premise were supplemented daily with 1.5 mg/kg of oral Microactive® CoQ10. Resting middle gluteal skeletal muscle biopsies were taken before and after 9 weeks of supplementation with each horse acting as its own control. Skeletal muscle mitochondrial electron transport chain combined complex CI+III activity assayed in triplicate was analysed with a paired Student's t-test ($P<0.05$). Complex CI+III activity increased by 40% after supplementation ($0.42\pm4.97E^{-05}$ vs $0.58\pm4.7E^{-05}$ pmol/min/mg protein normalised to mitochondrial abundance/g muscle, $P<0.01$). Functionally, increased skeletal muscle mitochondrial CoQ10 may result in more efficient energy production, delayed onset of fatigue during exercise, improved exercise training responses and enhanced recovery following intense exercise. This study is the first to demonstrate that daily oral supplementation of horses with CoQ10 increases skeletal muscle mitochondrial CoQ10.

Determinants of plasma ionized calcium concentration in Thoroughbreds and Standardbreds

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Ionized calcium (cCa, mmol/l) is the biologically active form and is negatively associated with plasma pH. Calcium fractions in plasma are in equilibrium and exist in three forms: free (≈50% of total), bound to proteins (≈40%), and complexed to anions (≈10%) such as L-lactate. Based on studies in humans and cattle, we hypothesized that cCa in exercising horses is dependent on plasma pH, plasma total calcium (cTCa) and total protein concentration, and blood L-lactate (cLact) concentration. A pH-corrective equation exists for human plasma, whereby measured cCa at measured pH is corrected to pH=7.40. The validity of this equation for equine plasma is unknown; we hypothesized that the pH-corrective equation for equine plasma differs from human plasma. To test our hypotheses, we characterized the *in vitro* plasma logcCa-pH relationship using CO_2 tonometry of 134 plasma samples from nine healthy adult Thoroughbreds. We subsequently investigated the *in vivo* plasma logcCa-pH relationship using 92 samples from eight trained Standardbreds undergoing a standardized exercise test. As hypothesized, the *in vitro* pH-corrective equation for equine plasma was different to that of human plasma, such that the slope (logcCa/pH)=-0.17 instead of -0.23. Multivariate regression indicated plasma cCa in exercising Standardbreds was positively associated with cTCa (partial-R^2=0.40) and negatively associated with cLact (partial-R^2=0.24) and blood pH (partial-R^2=0.18), indicating that clinically relevant amounts of ionized Ca are bound by lactate during exercise. We conclude that cCa in exercising horses depends on cTCa, cLact, and pH, and that hyperlactatemia has an important effect on decreasing cCa during exercise.

Effect of clipping and blanketing in exercising Icelandic horses

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The aim of this study was to investigate the effect of clipping and blanketing on the physiological response to exercise and recovery in Icelandic horses. Six horses in winter coat were studied before, during and 60 minutes after they performed a standardised exercise program. This was repeated four times, during which each horse received one of the following treatments: (1) unclipped; (2) unclipped and covered with blanket (wool) directly after exercise; (3) half-clipped; or (4) half-clipped and covered with blanket directly after exercise. Skin temperature of the left and right neck and hind legs, respiration rate and heart rate were recorded before, during and after exercise. Blood samples were taken at rest, after maximum exercise, after 2 min walk, and after 10 and 30 min in stable and analysed for haematocrit, total proteins, potassium, glucose, pH and pCO_2 and lactate. Data were analysed with Proc-Mixed Model in SAS. Results are presented as mean±SE. Heart rate increased to 173±2 beats/min after galloping but there was no treatment effect during recovery. There was no treatment effect on any of the blood parameters. Clipped horses had lower skin temperatures than unclipped horses before exercise. Horses with blankets after exercise had higher skin temperatures at the hindquarter that was covered with the blanket but not at the neck, compared to horses not covered with blankets. Clipped horses had significantly lower respiratory rates than unclipped horses 20 (32±8 vs 84±8, P<0.001) and 30 (16±8 vs 50±8, P<0.05) minutes after the exercise test. In conclusion, unclipped horses were forced to use an increased respiratory rate for heat dissipation during recovery.

Catecholamine responses to supramaximal hypoxic, normoxic and hyperoxic exercise in horses

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Thoroughbred horses achieve high aerobic capacities with severe exercise-induced hypoxemia due mainly to diffusion limitation; breathing hyperoxic gas alleviates hypoxemia and increases maximal oxygen consumption (VO_{2max}). Hypoxic training increases VO_{2max} more than normoxic training in well-trained horses. We hypothesized that breathing hypoxic gas would stimulate greater catecholamine release during maximal exercise because hypoxia induces more severe hypoxemia (arterial O_2 tension; 45±2 (SD) Torr) compared to normoxia (66±5 Torr). The purpose of this study was to quantify catecholamine responses at supramaximal exercise speeds in hypoxia, normoxia and hyperoxia in horses. Six trained horses ran in a crossover design up a 6% grade on a treadmill at supramaximal speeds sustainable for approximately 110 s (determined previously, approximately 115% VO_{2max}) breathing normoxic (NO, FIO_2=0.21) or hypoxic (LO, FIO_2=0.15) gas in random order. Horses also ran at the same speed, incline and run time as in NO breathing hyperoxic gas (HO_{NO}; FIO_2=0.29), and as in LO breathing normoxic gas (NO_{LO}, FIO_2=0.21). Horses ran on different days with VO_2 measured and atrial blood collected to measure epinephrine and norepinephrine concentrations. Data were analysed with ANOVA and Holm-Šidák pairwise comparisons. Oxygen consumption in NO was higher than in LO (166±11 vs 117±14 ml//kg/min). Catecholamines during supramaximal exercise (NO and LO) had higher concentrations than in HO_{NO} and NO_{LO}. Norepinephrine concentration in LO (29.3±14.9 ng/ml) was higher than in NO (20.1±7.6 ng/ml). Supramaximal exercise while breathing hypoxic gas induced more severe hypoxemia and may have stimulated greater norepinephrine secretion than supramaximal exercise while breathing normoxic gas in horses.

Creatine kinase reference intervals at rest and after maximal exercise in Standardbred racehorses

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Due to the high variability of data drawn from the literature, the aim of this study was to evaluate the effects of exercise on serum creatine kinase (CK) activity and to determine CK reference intervals (RIs) at rest and post-exercise in Standardbred racehorses. Data concerning history, physical examination, laboratory evaluation and ECG were collected retrospectively from a population of 258 Standardbreds in training that underwent incremental maximal treadmill exercise. Subjects with alterations potentially influencing CK were excluded. A reference sample of 194 healthy horses was eventually selected. Blood samples were collected 1 h before and 6 h post-exercise and analysed via a spectrophotometric method. Values were compared by Wilcoxon test for paired samples. The effect of age and sex was evaluated by Kruskal-Wallis and Dunn's post-test (P<0.05). RIs were determined following Clinical and Laboratory Standard Institute (CLSI) guidelines, approved by the American Society for Veterinary Clinical Pathology. Using a macroinstruction set for Microsoft Excel (RefValAdv), RIs were determined with a non-parametric method. A significant increase (P<0.0001) in CK activity post-exercise was observed. Partition by sex and age did not show any statistical difference, either at rest or post-exercise. In determination of RIs no outliers were identified. RIs ranged from 25-394 IU/l at rest and from 44-734 IU/l post-exercise. To our knowledge, this is the first study considering CK post-exercise RIs in racehorses using CLSI's guidelines and specific CK-related exclusion criteria. These RIs could be useful to discriminate between physiological and pathological post-exercise serum CK increases.

A retrospective study of 47 eliminated elite endurance horses requiring emergency treatment (WEG)

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The objectives were to study a group of elite horses competing internationally in endurance at the World Equestrian Games, eliminated during the ride and requiring emergency treatment, and to assess clinical, laboratory and therapeutic parameters, risk factors and correlations between findings. All horses (n=47) were removed from endurance competition and treated for a metabolic disorder. They were classified according to disorders presented, laboratory findings, clinical severity, individual performance record, age and distance at elimination. Standardized treatment regimens were assessed. Five horses that successfully completed the ride were included for comparison. Statistical analysis included descriptive statistics and nonparametric comparisons (ANOVA and Kruskal-Wallis). Clinical diagnoses identified included hydro-electrolytic imbalance (70%), ileus (64%), diaphragmatic flutter (21%), myopathy (9%), colic (9%), metabolic exhaustion syndrome (5%) and neurologic syndrome (2%). Elimination was significantly later with diaphragmatic flutter than with other disorders (P=0.037) and significantly earlier with myopathy cases (P=0.014).) Haematologic parameters were significantly increased in horses with myopathy compared to others. Biochemical modifications were all mild and mostly unhelpful for identifying sick horses. A large proportion of markedly dehydrated horses also developed paralytic ileus (40%). A strong trend for myopathic cases to develop ileus without dehydration was noted. Fluid management was similar between disorders, and a mean of 21.9±9 l was administered per horse, without significant differences between groups. Despite high elimination rate and ride difficulty, all horses were discharged successfully irrespective of diagnosis, highlighting the importance of early and aggressive management.

Activity affects blood lactate concentration during and after pace-races in Icelandic horses

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In Icelandic horse flying pace races, horses are supposed to pace as fast as they can. In each competition there are two attempts and between these attempts some recovery is allowed. The objective of this study was to investigate if blood lactate concentrations (Lac) were affected by the activity between and after the attempts. Six adult Icelandic horses used to pace-racing were studied in a simulated 100 m race. The study was performed as a crossover design with two treatments performed on two days, 7 days apart. The treatments were; walk/stand still (STILL) or slow trot (TROT) for 10 min, both between and after the attempts. The attempts were made in pairs with one horse on each treatment. Blood samples were collected after the finish line in both attempts and 10 min after each attempt and analysed for Lac. ANOVA (MIXED procedure) was performed (P<0.05). The average velocity during pacing was 11.9 m/s. There was no difference in Lac after first attempt (STILL: 9.9±1.8 vs TROT:8.1±1.7 mmol/l). After first recovery, Lac was higher at STILL than TROT (10.0±1.8 vs 5.1±1.7 mol/l, P=0.02). There was no difference in Lac after the second attempt (STILL: 12.4±1.8 vs TROT: 10.7±1.7 mmol/l) but after the second recovery, Lac was higher at STILL than TROT (14.4±1.7 vs 7.6±1.7 mmol/l, P<0.01). This study shows that active recovery lowers Lac before the second attempt. These results have practical implications since horses are traditionally standing still or walking between attempts.

Assessing the ammonia response of Thoroughbreds to strenuous exercise with a point-of-care analyser

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Plasma concentrations of ammonia (NH_3) increase dramatically with exhaustive supramaximal exercise. However, studies of this NH_3 response have been hampered by assays that only use plasma, and time sensitive alterations in plasma [NH_3] due to the nitrogen in air. The recent advent of a hand-held point-of-care analyser that can measure whole blood [NH_3] represents an opportunity to conduct field studies of the NH_3 response of horses to exercise. We aimed to investigate the relationship between blood [NH_3] and exercise speed and distance in Thoroughbred racehorses. 67 jugular samples were obtained from 59 Thoroughbreds within 15 min of breezing or racing over 800 to1,600 m and immediately analysed to determine blood [NH_3] using a hand-held analyser developed for this purpose and previously shown to have acceptable linearity and precision. Linear regression analysis assessed correlations between individual blood [NH_3] and speed for each distance, between [NH_3] and exercise distance, and between pooled [NH_3] and speed for combinations of sequential distances. Significance was set at $P<0.05$. Mean±SD blood [NH_3] ranged from 152±49µmol/l for 800 m to 415±119 µmol/l for 1,200 m. Blood [NH_3] was well correlated with distance for 800-1,200 m ($P<0.001$; $r^2=0.53$) and moderately correlated with speed for 800-1,100 m ($P=0.05$; $r^2=0.28$), but not with speed or distances from 1,200-1,700 m ($P=0.25$; $r^2=0.02$). [NH_3] and speed were not correlated at any specific distance. Post-exercise blood [NH_3] is related to exercise speed over 800-1,100 m and distance from 800-1,200 m. It was easy to measure [NH_3] with the hand-held device. This might prove useful in monitoring responses to training and racing.

Radiotracer flux of 24Na, 42K and 99Tc-DTPA in horses during exercise and recovery

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The purpose was to determine the appearance of fluid and electrolyte radiotracers in sweat during exercise with three supplement conditions. Four horses received IV infusion of ^{99}Tc-DTPA (extracellular fluid – ECF marker) and ^{24}Na-labeled water (1 l) or electrolyte supplement (ES, 1 l and 3 l) by nasogastric intubation (NI) 1 h before start of exercise. There were no differences in sweat outcome measures between the three trials, therefore trial data were combined. In separate trials, four adult horses received ^{42}K-labeled water (8 l) or ES (8 l) by NI 1 h before start of exercise. Data were assessed using repeated measures ANOVA. The ^{99}Tc counts appearing in sweat (by 10 min) averaged ~2% of plasma dpm/ml, suggesting movement of DTPA through paracellular pathways, and could not be used to calculate sweating rate. The sweat [Na^+] was consistently less than that of plasma although specific activity (SA) of ^{24}Na in sweat (by 10 min) equalled that in plasma, indicating that sweat sodium is a filtrate of ECF. ^{24}Na was used to estimate Na^+ flux into sweat and the values, though variable, agreed with measured sweat [Na^+]. The sweat [K^+] were consistently much greater than in plasma, and SA of ^{42}K in plasma (by 5 min) averaged 1/13th that in sweat. This agrees with a mechanism for active sweat K^+ secretion. Sweat [K^+] was greater ($P<0.001$), sweat K^+ SA was less ($P<0.001$), plasma K^+ SA was less ($P<0.001$) with ES than water. Sweat K^+ flux was less ($P<0.001$) in ES (14.4±4.1) than water (44.8±3.5 mEq/l/h). It is concluded that fluid and electrolytes ingested 1 h prior to exercise appear in sweat, and that sweat is an ECF filtrate to which K^+ has been secreted.

Changes in cytokine expression in blood leukocytes after cross country in eventing horses

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In endurance and race horses, the efforts during elite competitions (long distance rides and flat races) result in exercise-induced acute phase response (APR), characterized by increases in serum amyloid A (SAA) and proinflammatory cytokines levels. In the horses competing in three-day events, slight changes in SAA have been described, suggesting APR, but cytokine levels have never been measured. The aim of this study was to determine how the expression of cytokines, involved in the regulation of APR, changes in elite eventing horses. Blood samples were taken from competing horses (n=8), before cross country, immediately after, and 4 hours later. The expressions of IL-1, IL-6, IL-10, IFNγ and TNFα were measured by QT-PCR. Spearman correlations among changes (x-fold) of variables were analysed using Statistica 13.1. The expression of proinflammatory (IL-1, IL-6, IFNγ) and anti-inflammatory (IL-10) cytokines after cross country increased even 100 times, but large individual variations occurred. Strong (r=0.83, $P \leq 0.05$) positive correlations have been observed between the changes in IL-1 and IL-6 expression after exertion and 4 hours later. The changes in IFNγ correlated positively with IL-1 (r=0.89, $P \leq 0.05$) and IL-10 (r=0.67, $P \leq 0.05$) expression. The increases in proinflammatory cytokines expression suggest that in eventing horses the type 1 APR is promoted (IL-1 as a primary signal). The simultaneous increase in IL-10 may indicate the condition preventing clinical onset of APR. It can be postulated that exercise-induced APR similar to that reported previously in race and endurance horses is likely to occur in eventing horses.

Physiotherapy, Rehabilitation and Equitation science

Initial testing of a computer-integrated weight compensation system for rehabilitation of horses

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Management of severely injured or neurologic horses is challenging, with ambulatory support limited to static lifts and rescue slings. The aim of this study was initial testing and adjustment of a novel computer-integrated dynamic lift system, including measuring effects of increasing weight compensation (i.e. load reduction) and time attached to the lift. This automated system was developed to advance rehabilitation techniques, allowing for controlled mobility and varying load carried by the horse with independent front-hind and side-to-side support. Two healthy Thoroughbred horses were studied using the Anderson Sling. The lift was programmed to respond to weight and movement of horses. Weight compensation (% bodyweight) was incrementally increased, for front and hind limbs, to maximum percent tolerated, based on heart/respiratory rates and behavioural scoring. The time attached to the lift was then incrementally increased at maximum tolerated weight compensation. Measures included heart/respiratory rates, behavioural scoring, muscle enzyme activity and blood flow to distal limbs. Results were analysed descriptively. Aversion behaviour was observed at front and hind end weight compensation >20% and 5%, respectively. Average maximum time attached to the lift was 2.25 hours. After 60 minutes, respiratory rate increased >20/minute, reaching 60/minute and 36/minute, respectively, with shallow breathing. Other measures remained normal. In conclusion, dynamic lifting was successful for weight compensation and mobility during lift support. Complications included aversion behaviour and respiratory distress at >20% weight compensation, likely caused by the Anderson Sling. To address these limitations, a new rehabilitation harness better suited for long-term use is under development.

A comparison of mean peak rein tension in Western & English riding

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Inappropriate rein cues and painful bit pressure jeopardize horse welfare. Using objective methods to quantify rein tension in daily training might assist riders in better judging their own rein contact. This study investigated the difference between rein tension in Western (W) and English (E) riding. A portable rein tension device was used in 106 combinations of 65 riders and 88 horses performing standardized tasks in E (51 rides) and W (59 rides). Mean rein tension of each task was compared to mean peak tension (consecutive maxima per task) with mixed-model analysis. The magnitude of peak rein tension varied between and within the different gaits and transitions (P<0.0001). It was overall between 0.1 N (sitting trot: 10.1±0.7 N peaks vs 10.02±0.7 N mean) and 2.2 N (canter-walk: 14.6±2.2 peaks vs 16.8±2 N mean) higher than mean rein tension. English riders applied higher mean peak rein tension overall and within different gaits compared to Western riders (walk: 12.9±0.6 vs 7.5±0.4 N; sitting trot: 19.6±1 vs 9.8±0.5 N; canter: 25.5±1.3 vs 13.4±0.6 N, respectively, all P<0.0001) and peak tensions also differed within the specific disciplines (show jumping: 23.1±5.1 N; dressage: 17.3±2.9 N; leisure riding: 17.6±3 N in E; reining: 12.9±2.1 N, cutting: 14.8±2.4 N, all-round: 12.4±2 N in W, P<0.0001). Peak tensions showed minimal difference between manoeuvres and directions. Riders reached higher peak tension in the inside rein, except when riding counter-clockwise on a straight line (P<0.0001). Compared to earlier results on mean tension, peak rein tension exhibited higher mean values, but both seem to be mainly influenced by gait, riding style and discipline.

Reducing the pain from overriding dorsal spinous processes increases multifidus cross sectional area

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To evaluate changes in cross-sectional area (CSA) of the multifidus muscle following treatment for overriding dorsal spinous processes (ORDSP), 45 horses with chronic histories of topline muscle atrophy, clinical evidence of back pain (dorsiflexion in response to digital spinal pressure) and radiographic evidence of ORDSP had the CSA of the left and right multifidus muscles measured at three vertebral positions (T15, T18 and L2) before and after treatment. Thirty-three horses underwent interspinous ligament desmotomy and 12 horses had methylprednisolone acetate injected into the affected spaces. All horses followed a similar exercise program. The multifidus muscle CSA's were compared statistically using independent and paired sample t-tests. No difference in pre-treatment CSA was present between the groups, nor was there left vs right asymmetry within horses. The CSA significantly increased at all measurement points after both surgical and medical treatment. The pre-treatment CSA (mean±SE) of 15.3±1.3 cm^2 increased 19% to 18.3±1.2 cm^2 after treatment (P<0.0001). The rate of CSA change was not significantly different between treatments, nor was the proportion of horses with reduced dorsiflexion in response to digital palpation. Surgically treated horses displayed a greater increase in CSA (P<0.0001), but also had longer average time to reassessment (56 vs 26 days). Greater pre-treatment multifidus atrophy was associated with a greater post-treatment CSA increase. Reduced dorsiflexion in response to pressure preceded increased multifidus muscle CSA, suggesting a link between improved spinal comfort and functional health. Serial multifidus CSA measurement provided an objective assessment of treatment response.

Effects of pulsating infrared and red monochromatic light on swelling and wound healing in horses

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Light-emitting diodes (LEDs) are commonly used for treating a variety of disorders in horses. The purpose of this study was to investigate how treatment with an LED device emitting pulsating infrared (λ≈956 nm) and visible red light (λ≈637 nm) affects swelling and wound healing time. A skin wound (2 cm diameter) was created on each side of the neck in eight healthy horses. One randomly chosen wound received active light treatment and the other served as control. Treatment duration was 4 min 40 s (infrared light 185 s, red light 95 s) and was performed once daily, five days a week for four weeks. The irradiance was 6.4 mW/cm^2 for infrared and 2.3 mW/cm^2 for red light. The wounds were photographed (wound area evaluated by digital planimetry) and the degree of swelling (cm) was assessed with diagnostic ultrasound day 0, 1, 2, 3, 4, 7, 14, 21, 28 and 35. Data were analysed with t-test and ANOVA (P<0.05). The wound area (P=0.20) and degree of swelling (P=0.99) did not differ between treated and control groups on any day. Additionally, wound areas were assessed daily with a wound measurement ruler by an assessor blinded to the treatment and time to total healing was registered. There was a significant difference (P=0.026) in healing time between control wounds (49.0 days, 95%CI=35.4-62.6) and treated wounds (51.8 days, 95%CI=38.7-64.8). The results showed no positive effect of treatment with pulsating infrared and red visible light on swelling and wound healing in experimental skin wounds, compared to no treatment.

The effect of kinesiology tape on the walking gait of normal horses

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Kinesiology taping (KT) has become popular in both human and veterinary rehabilitation, yet there is a paucity of studies specifically focusing on the use of KT in horses. Preliminary studies within human research indicate that KT may improve joint proprioception and muscular strength. The aim of this gait analysis study was to compare horizontal, shear and vertical forces, and corresponding moment components in a group of sound horses at walk. Recordings were taken under three taping scenarios; without, with tape applied to the right biceps femoris muscle, and with tape applied to the right hind pastern in order to test if the forces/moments recorded varied between different tape applications. Five Standardbred mares were walked over the Advanced Mechanical Technology Incorporated force plate in each scenario until a total of ten valid trials were recorded. A trial was considered valid when the right hind and right forelimbs made contact with the force plate respectively (a lateral sequence). From the ten valid trials recorded, graphical data were generated, and the best five graphs were analysed for each scenario. The amplitudes for all force and moment components were recorded, along with time of foot lift-off and foot strike. Data were collated and prepared for statistical analysis. A 2-way ANOVA was performed (P<0.05) and no statistical difference was identified between each scenario. A number of reasons may explain these results including inadequate stretch applied to the tape, and a lack of proprioceptive deficits in the sample group. It is recommended therefore, that further research with KT be undertaken in horses.

Analysis of faults in International Showjumping Competition

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It is commonly believed by riders and trainers that faults in showjumping are not random but are associated with particular types or location of fences. This study aimed to characterise faults as defined by the FEI (knocking down a fence pole/plank, displacing an obstacle, a foot landing in a water jump, refusal or 'run-out'). All rounds of horse and rider combinations competing in the 2nd Round of the FEI Nations Cup 2017 competition in European Division 1 at 8 different 5* outdoor events were reviewed. Fences were classified by jumping effort (incremental), by type (e.g. upright, oxer, etc.), by approach line (straight, left-rein, right-rein) and by direction (in relation to the collecting-ring; away, towards, across). Statistical approach used correlation, chi-squared and multi-variable models. 2,550 jumping efforts were analysed. The most common faults were knock-down (5.5%), time penalties (0.8%), fault at a water jump (0.3%), and refusal/run out (0.2%). There was a linear relationship between jumping-effort number and number of fences knocked-down (r^2=0.7; P<0.001). There were 2.8 times more knock-downs for the second half of the course (efforts 9-15) compared with jumping efforts 1-7 (P<0.05). Faults were more common (percentage of attempts) when fences were jumped straight-on (7.9%) compared with either at a slight right or slight left approach (<45° from previous fence; 6.2%) or on a left or right rein (>45° from previous fence; 3.8%; P<0.001). This analysis suggests that for these competitions, faults were not randomly distributed.

International survey of veterinarians using rehabilitation modalities in horses

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To define which biologic, electrophysical and other modalities are used in horses for injury or performance issues, a questionnaire regarding 38 modalities was distributed to eight veterinary groups. 305 responses were obtained from >10 geographic regions; 76% from private equine practice or private referral hospitals, 14% from university hospitals or clinics, 8% from private mixed practice, and 2% from veterinary rehabilitation centres. Most respondents were in the USA (60%), Europe (26%) and Canada (6%). Respondents primarily worked in hunter-jumper (27%), dressage (16%), and pleasure riding (15%); predominantly with Warmbloods (40%), Thoroughbreds (20%) and Quarter Horses (17%). Handwalking (97%), therapeutic shoeing (96%), ice (95%), compression bandaging (90%), PRP (87%), therapeutic exercise (84%), IRAP (81%), stretching (83%), and cold water hydrotherapy (83%) were most commonly utilized, as were heat (78%), massage (69%) and acupuncture (68%). Electrophysical therapies were predominantly applied to tendon and ligament injuries and soft tissue modalities were most often applied for performance purposes, injuries of the neck or back, or muscle strain. Injectable modalities were almost solely administered by veterinarians, for treatment of tendon and ligament injury or after arthroscopic surgery. Other modalities were variably applied by veterinarians, technicians, farriers, physical therapists, trainers and other entities for different performance and injury scenarios. 33% of respondents consulted with physical therapists, who were common providers of massage (26%), chiropractic (18%) and taping (16%). A range of rehabilitation modalities are used in athletic horses, administered by a variety of personnel. Further investigation is indicated to better define their delivery, efficacy and effects.

Laterality's influence on symmetry and time shift between left and right rein tension peaks

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This study investigated the temporal and quantitative symmetry of rein tension peaks (consecutive maxima per task) in Western (W) and English (E) riding using a portable rein tension device with 65 riders (14 left-handed (LH), 49 right-handed (RH), 2 ambidextrous) and 88 horses performing standardized riding tasks. Equine laterality was assessed based on (1) horse's preferred side for riding as validated in earlier studies (41 right-lateral (RL), 35 left-lateral (LL), 12 without side-preference) in E (51 rides) and W (59 rides) and (2) horse's lateral displacement of the hindquarters in relation to the median plane while standing. Mixed-model analysis revealed a smaller time shift of peaks between the left and right reins (TSLR) for RH (0.06 s) vs LH (0.1 s, P=0.01) and combinations of riders with LL (LL/LH 0.04 s., LL/RH 0.05 s. vs RL/RH: 0.06 s., RL/LH: 0.07 s., P=0.0007). Larger TSLR was detected counter-clockwise (circle: 0.15 s., straight: 0.04 s., clockwise, circle: 0.01 s. straight: 0.02 s., P<0.0001) and in horses with right-displaced hindquarters (0.04 vs 0.02 s., P=0.046). The mean difference of tension between left and right rein determined continuously throughout the session was not significant (P>0.05). Smaller TSLR was detected in W (0.02 s. vs E: 0.05 s., P=0.014) and familiar horse-rider combinations (0.02 s. vs unfamiliar: 0.05 s., P=0.005). Both also revealed lower mean difference (W: 2.6 N vs E: 7.5 N, P<0.0001; familiar: 4.1 N vs unfamiliar: 4.8 N, P<0.0001). Equine and human laterality influenced the temporal, but not the quantitative symmetry of the left and right rein. Western riding and well-acquainted horse-rider pairs showed improved temporal and quantitative symmetry of rein tension.

Acute effects of equine therapeutic massage

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Massage is a commonly used therapeutic method for relief of soreness, and for general and muscle relaxation. The purpose of the present study was to evaluate the acute effects of therapeutic massage of lumbar muscles on equine behaviour, cortisol concentration, mechanical nociceptive threshold (MNT), residual muscle tension and blood flow. Nine healthy horses were included in a prospective, randomized, crossover study. The study protocol was as follows: baseline (20 min); massage or no massage (controls) (30 min); and post-massage (20 min). Data were analysed with ANOVA (P<0.05). Ethograms were used to assess the frequency of behaviour indicating different degrees of relaxation, and salivary cortisol levels to assess stress. Algometry, electromyography and near-infrared spectroscopy were used for measurements of MNT, residual muscle tension and blood volume, respectively. Results showed a lower frequency of relaxation during massage (P<0.05) and a higher frequency of relaxation during the post-massage period (P<0.0001), compared to controls. There was a non-significant decrease in salivary cortisol concentration for four out of five horses, and no significant differences in residual muscle tension and blood flow between massaged horses and controls. The results partially support the existing evidence of massage having a relaxation effect. Further studies are needed to fully describe the acute and long lasting effects of equine therapeutic massage.

Development of a novel harness system to aid in rehabilitation of horses

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Development of a harness to aid in rehabilitation from laminitis and musculoskeletal injuries is needed because serious complications (e.g. compromised breathing) arise from longer-term use of rescue slings. This study's objective was to evaluate equine biomechanics, and physiological and behavioural measures for development of a rehabilitation-specific harness when used with a novel computer-integrated dynamic lift system. This dynamic lift reduces the load the limbs carry, either front to back or side-to-side. The rehabilitation prototype was made of cotton/nylon with sheepskin inserts, forming a blanket-like pattern. High-strength/low-weight strapping, integrated into the fabric, circumferentially attaches the load-bearing structures of the horse to the lift. The prototype was tested to a load of 600 kg. In an adult horse, the harness allowed for 40% load reduction from both front (125 of 303 kg [60% of 506 kg]) and hind (80 of 203 kg [40% of 506 kg]) limbs before complications (spreading and bowing of the front legs) occurred. The addition of an H frame and a figure eight pattern to the forelimb support improved posture and achieved greater reduction in load (46% [140 of 301.2 kg]). Abnormalities in respiratory rate or pattern were not observed. Rehabilitation harness design considered normal load-bearing structures of the horse, distributing pressure over a greater surface area on the chest, shoulders and legs while removing it from the thorax and abdomen. Further testing will include the use of electronic sensors to detect high-pressure, high-temperature, high-moisture areas, adjusting the design further for improved horse-comfort enabling longer-term use during rehabilitation.

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