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Comparison of steroid hormone response to exercise between humans and horses.

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The goal of training in horses such as in humans is to provide a stimulus for specific adaptation that will result in improved athletic performance. But the physiological adaptation to training could be impaired by overtraining. There is a wide range of biochemical, haematological and physiological markers reported to be stress indicators in human athletes as training load is progressively increased. Fewer numbers of biochemical parameters have been studied in horses. The comparison between human and horse athletes’ hormonal response to training allows verifying to what extent there is a similarity in the normal hormonal response to training and to what extent the hormone measurement could be used to prevent overtraining. The steroid hormone response to exercise and training has been extensively studied in humans. It is well established that intensive endurance training results in a decrease in gonadal hormone production in males and is associated with a stimulation of cortisol production during exercise period so that the Testosterone:cortisol ratio was proposed to monitor the training adaptation. It is suggested that the Testosterone:Cortisol ratio variations are representative of anabolic and catabolic states and could be reliable with the metabolic and behavioural changes observed in overtrained humans. The hypothalamo-pituitary-adrenal (HPA) axis is activated by exercise and the level of response is under influence of exercise duration and intensity. It is proposed that the HPA axis state at rest and the exercise response could be an index of training adaptation. The HPA axis plays a pivotal role in the response to acute exercise and adaptation to training. Training in humans reduces HPA axis response to a same absolute workload. This was interpreted as an adaptation which limits prolonged exposition of tissue to high glucocorticoid level. In humans, however, data dealing with cortisol response to training are conflicting. On the one hand significantly elevated resting plasma cortisol has been detected in overtrained runners with impaired performance whereas declines or increases in plasma cortisol were observed between overreached swimmers. More recently, it was demonstrated that overtraining could influence the inactivation of cortisol into cortisone as shown by an elevation in urinary cortisol/cortisone ratio (Gouarné et al 2005). In humans, it has been established that physical training enhances adrenal sensitivity to adrenocorticotropine (ACTH). In horses, Golland et al (1999) demonstrated that maximal exercise increases plasma cortisol and testosterone as in humans. There is no data that indicates a decrease in plasma testosterone under the effect of exhaustive prolonged exercise or intensive training in horses. The HPA axis has only been documented as hormonal markers to training response. It was shown by Alexander et al (1991) that the stimulation of the HPA axis under the effect of exercise in horses results in both ACTH and argininevasopressin (AVP) release in the pituitary venous effluent. This observation suggests that the plasma osmolality increase produced by exercise could stimulate the magnocellular nuclei at the hypothalamic level. In humans, the cortisol rise is also influenced by corticotrophin-releasing factor (CRF) changes. Conflicting data have been reported on the relationship between training status and cortisol response to exercise in horses as in humans. One study was designed to evaluate the diagnostic value of plasma ACTH and cortisol measurement in assessing the training status in horses (Marc et al 2000). The data confirmed that training decreases cortisol response for a same absolute workload. Stimulation of cortisol secretion by an ACTH injection results in lower plasma cortisol concentration in trained horses than in untrained horses.

The comparison of human and horse HPA axis response to exercise confirms that the adrenal hormones could be used as a reliable index for training adaptation. Meanwhile data about the balance between anabolic and catabolic hormone changes under the effect of training including gonadal steroids but also the somatotropic axis are lacking in horses.
Relationships between exaggerated symptoms of estrus and sex hormone profiles in athletic mares: a retrospective study of 100 cases

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In some athletic mares strong or abnormal sexual behaviour are associated, according to their riders, with a failure to perform at their best or with difficulties to be handled during estrus-like periods. The aim of the present study was to assess a relationship between abnormal sexual behaviour, in sport mares, and sex hormone levels.

One hundred mares were selected by different equine practitioners in accordance with clinical signs of this syndrome. Blood samples were collected every week, 4 times in each mare: estradiol, progesterone, prolactin, testosterone, 17-OH-progesterone and androstenedione were assayed in plasma. Commercially available kits were used. All assays used were previously validated for use in horses in our laboratory.

Mares ranged from 1 to 20 y of age (mean 7.7 ± 4.1). A decreased performance was reported in 47% cases. Abnormal behaviour was reported to be permanent in 45% of mares and intermittent (“cyclic”?) in other mares. The clinical signs reported included squealing (34.4%), aggressiveness (54.0%), anxiety (52.2%), escape (64.6%), and urination (37.7%).

Sex hormone levels showed the following: an ovarian cyclic activity in 82% of mares, a continuous luteal phase in 7%, a seasonal anestrus in 2% and abnormal ovarian activity in 9%. Ovarian cyclicity was found in 83% of mares described with permanent abnormal sexual signs. Only one mare had an abnormal high level of estradiol, she had an ovarian tumour. Abnormal levels of testosterone were measured in 8% of mares. When prolactin levels were considered, mares could be divided into 3 groups: normal level 45% of mares, high level 18% and very high level 37%. There were no significant correlations between prolactin levels and other hormone levels, the characteristics of the mares (age, breed, activities...), ovarian cyclicity or clinical signs except anxious behaviour. Abnormal estrus-like signs in athletic mares were not correlated with ovarian activity and sex hormone secretion in the majority of cases. Hyperprolactinemia is noted in 55% of mares with this syndrome. Is prolactin just a sign or is it implicated in the pathogenic process?
The effect of long-term exercise on glucose metabolism and peripheral insulin sensitivity in Standardbreds

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Introduction

Twelve Standardbred geldings (1.5 years old) were selected for a longitudinal training study in order to assess the effects of training on glucose metabolism and peripheral insulin sensitivity.

Material and methods

The horses were acclimatised to running on a high-speed treadmill for 4 weeks (phase 1) followed by training for 18 weeks with an alternating endurance (~ 60% HRmax) high intensity training program (~ 80% HRmax)(phase 2). Training frequency was 4 days/week. Two matched Standardbreds served as untrained controls. At the end of both phases, a euglycaemic hyperinsulinaemic clamp test was performed. A priming dose of 45 mU of insulin/kg BW was given iv followed by an insulin infusion at a constant rate of 6 mU/kg BW/min. Peripheral insulin sensitivity was calculated as the ratio of the glucose metabolism rate to the plasma insulin concentration. Statistical analysis was performed using the Huynh-Feldt test for repeated measures analysis for the differences between both periods and the Mann-Whitney test for differences between trained and untrained horses. P values < 0.05 were considered significant. The results are presented as mean ± SD.

Results

Complete data were obtained from six horses. Glucose metabolism rate after phase 1 (23.6 ± 5.82 µmol/kg BW/min) was not significantly different from the values after phase 2 (24.5 ± 7.20 µmol/kg BW/min). Peripheral insulin sensitivity also did not change significantly following training (0.0153 ± 0.0073 and 0.0086 ± 0.0034, respectively). However, a trend is seen for increasing peripheral insulin sensitivity for the trained Standardbreds.

Conclusion

Preliminary results indicate that long-term training in Standardbreds neither changed glucose metabolism nor insulin sensitivity.
Short term training increases insulin sensitivity, Glut-4

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Introduction

Expression and Glycogen Synthase Activity in Standardbred Horses Evidence regarding the mechanism for exercise training-induced enhancement of insulin sensitivity in horses is limited. In this study, it was hypothesized that short term training (STT) would result in enhancement in insulin sensitivity in conjunction with an increase in muscle Glut-4 protein expression (GLUT4) and glycogen synthase (GS) activity, but that these training associated changes would be transient, returning to baseline after 5 days of detraining (DT).

Material and methods

Seven mature Standardbred horses completed STT consisting of 7 consecutive days of 45 min of treadmill exercise at a speed that elicited 50-55% of pre-training aerobic capacity (VO2peak). Insulin sensitivity was determined by mean glucose infusion rate (GIR) during a 120-min euglycemic hyperinsulinemic clamp (EHC) performed before (-3 days) and at 1 and 5 days after STT. VO2peak was measured before (-1 day) and at 1 and 6 days after STT.

Results

STT resulted in a 9% increase in mean VO2peak (mL/kg/min) (untrained (UT):139.9 ± 11.5 vs. trained (T):152.8 ± 17.0, P < 0.05) that was maintained in DT (152.0 ± 14.7). Mean GIR (mg/kg/min) was increased more than 2-fold following STT (UT: 6.6 ± 3.9 vs. T: 14.4 ± 2.3; P < 0.05), and was maintained above UT values in DT (10.3 ± 3.1, P < 0.05). GLUT4 was increased more than 10 fold in T and DT (P < 0.001). Pre-EHC GS activity (I form) (nM/hr/mg) (T: 99.0 ± 23.6; DT: 90.5 ± 31.9) and GS activity ratio (T: 0.44 ± 0.10; DT: 0.43 ± 0.10) respectively were increased (P < 0.05) in T and DT when compared to UT (53.9 ± 16.3; 0.28 ± 0.05).

Conclusion

Short-term training resulted in an increase in insulin sensitivity with associated increases in Glut-4 protein expression and glycogen synthase activity and these enhancements were still evident after 5 days of detraining.
Low dose exogenous erythropoietin elicits an ergogenic effect in standardbred horses

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Introduction

Eight healthy, unfit mares (4-10 yrs, ~500 kg) were used to test the hypothesis that recombinant human erythropoietin (EPO) administration would alter red cell volume (RCV), aerobic capacity (VO2max), and indices of anaerobic power.

Material and methods

Mares were accustomed to the laboratory and experimental protocols and randomly assigned to a control (CON, n = 4; 3 mL saline 3 times/wk for 3 wks) or EPO group (EPO, n = 4, 50 IU/kg rhuEPO/3 mL saline; 3 times/wk for 3 wks). Exercise tests (GXT) were performed on a treadmill (6% incline), 1 wk before and 1 wk after treatment. The GXT started at 4 m/s, with a 1 m/s increase every 60 s until the horse reached fatigue. Oxygen uptake was measured via an open flow indirect calorimeter. Blood samples were collected before, during (each step), and 2 and 15 min post-GXT to measure hematocrit (HCT), hemoglobin concentration (Hb), blood lactate concentration (LA), and plasma protein concentration (TP). Plasma volume (PV) was measured using Evans Blue dye. Blood volume (BV) and RCV were calculated using HCT from the 8 m/s step of the GXT.

Results

There were no alterations (P > 0.05) in any parameters in CON horses. By week 3, EPO produced increases (P < 0.05) in resting HCT (37±2 vs. 51±2), and Hb (37%). RCV (26%) and VO2max (11.6%) increased, but BV did not change (P > 0.05) due to decreased PV (-11%, P < 0.05). No differences (P < 0.05) were detected for TP, LApeak, VLA4, run time, Vmax, or velocity at VO2max.

Conclusion

Low dose rhuEPO administration increases RCV and aerobic capacity without altering anaerobic power.
Effect of exercise on innate immune responses in Thoroughbreds

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Introduction

Metabolic acidosis is a well-documented consequence of rigorous exercise in horses. In recent years, alterations in extracellular pH have been associated with changes in function of the innate immune system in other species. The objective of this study was to evaluate the effects of metabolic acidosis induced by a standardized exercise test on the production of reactive oxygen species and phagocytosis of bodipy-labeled E. coli and S. aureus by equine peripheral blood leukocytes.

Material and methods

Nine thoroughbred horses (5.1 ± 1.4 yrs) acclimated to a standardized exercise test were used in this study. Blood samples were collected before exercise, and at 8 m/s, 10 m/s, fatigue, and 2 hours after fatigue to monitor concentrations of metabolites and as a source of leukocytes for in vitro studies.

Results

At fatigue, the horses achieved an average V02MAX of 146.8 ± 11.3 mL/kg/min and a heart rate of 207.5 ± 8.9 beats/min. Phagocytosis of E. coli by neutrophils and monocytes was significantly greater than phagocytosis of S. aureus before exercise, at each exercise time point, and 2 hours after exercise (P < 0.0001). Exercise decreased the in vitro production of reactive oxygen species (ROS) by leukocytes stimulated with phorbol myristate acetate (PMA) at 10-6 M. PMA-induced production of ROS was higher at failure and two hours after exercise stimulation than before exercise, at 8 m/s, and at 10 m/s (Exercise by ROS; P < 0.0001).

Conclusion

Respiratory infections occur commonly in racehorses, suggesting that metabolic acidosis could be a predisposing factor to these infections. Over training and failure to treat exercise-induced metabolic acidosis may reduce innate immune and cellular responses to pathogens.
Investigation of blood oxidant/antioxidant markers in healthy competition horses: effect of discipline and gender

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Introduction

The aim of this study was to assess whether discipline- and gender-related differences of blood oxidant/antioxidant markers exist in competition horses.

Material and methods

505 healthy horses (337 thoroughbreds, 116 jumping horses, 40 standardbreds, 12 eventing horses; 180 females, 245 stallions, 79 geldings) underwent oxidant/antioxidant blood marker determination. VitC, VitE, α-carotene, lipophilic antioxidant capacity (ACL), glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), Selenium (Se), Copper (Cu), lipid peroxides (Pool), and oxidised proteins (Protox) were determined as well as creatine phosphokinase (CPK), lactate dehydrogenase (LDH), haemoglobin (Hb) and white blood cells (WBC). A linear model allowing exclusion of feeding-related differences assessed the effect of discipline and gender. P < 0.05 was considered significant.

Results

The thoroughbreds showed the highest values of VitE, α-carotene, ACL, GSH and Hb, whilst standardbreds had the highest values of VitC, Pool, CPK and LDH. Jumping horses had the lowest LDH and WBC values. Eventing horses had the highest Protox and the lowest GSH values. Females had significantly lower VitE, α-carotene, GSH and ACL values and significantly higher Pool values. The stallions had the highest Hb and WBC values, whereas the geldings had the lowest values of SOD, Pool, Hb and WBC. Correlation analyses were positive and significant between VitE and α-carotene, VitE and GPx, VitE and ACL, Se and GPx, Cu and Pool.

Conclusion

These results indicate that the blood oxidant/antioxidant status of horses is influenced by discipline and gender. The correlation analyses suggest synergistic relations between antioxidants. These findings are interesting for defining the specific needs for antioxidants and vitamins in competition horses.
Metabolic fate of 13C-lactate post-exercise

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Introduction

Excess post-exercise oxygen consumption (EPOC) represents a portion of the metabolic costs incurred during an exercise period. After exercise, accumulated lactate is cleared by oxidative pathways; thus a portion of EPOC is due to the oxidative removal of lactate. The purpose of this study was to quantify the contribution of this lactate during metabolic recovery from a brief bout of maximal exercise in horses–as part of a larger examination of the influence of body mass on lactate metabolism.

Material and methods

6 horses (3 TB, 3 Arabs: 480 ± 58 SD kg) performed a 60-sec exercise bout (9.2 to 9.9 m/s) on a 10% incline. Oxygen consumption was measured continuously using an open flow system. Blood lactates were measured at timed intervals. Prior to exercise, 13C-lactate was injected (2.2 mg/kg, IV). Expired gas samples were collected and analyzed for 13C over three 30-min time periods: rest, 0-30 min (included exercise), and 30-60 min. Data were analyzed with a repeated measures ANOVA (P < 0.05).

Results

VO2 peak averaged 134 ± 8 mls/kg/min. Lactate values peaked (13.7 ± 3.6 mM) 10-min post-ex, decreasing by 55% during recovery. 13C data indicate 20.4% of the total lactate pool is removed oxidatively, suggesting a glycogenic fate for the majority of lactate post-exercise.

Conclusion

The contribution of lactate oxidation to EPOC was relatively small and is less than in humans. These data are consistent with an allometric pattern of EPOC and post exercise lactate metabolism in several mammals – a decreasing reliance on oxidative removal with increasing body mass.
Compromise of exercising muscle blood flow by environmental heat stress in the horse

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Materials and methods

Radioactive microspheres were used to measure cardiac output and blood flow to most major tissues in four ponies at rest and near the end of 30 min moderate exercise (3-3.8m/s;~30% VO2max) in thermoneutral (TN) (dry/wet bulb = 16/12 C) and mildly hot (MH) conditions (41/27 C).

Results

In response to exercise in both TN and MH, there were increases in cardiac output (to ~35 L/min) and, mean arterial pressure (MAP) (120 mmHg) and oxygen consumption (3.2 L/min) and a decrease in PaCO2, with the hypocapnia greater in MH (37.8 vs. 28.3 mmHg). Central venous pressure (CVP) decreased with exercise in MH (to near zero) but was otherwise unaltered. Skin blood flow (body and limbs) increased 3-fold at rest in MH vs no change in TN. During exercise in TN skin blood flow increased ~33%, but decreased by ~5% during exercise (vs. rest) in MH. There was an increase in blood flow to respiratory and exercising muscles and myocardium during exercise in TN and MH. However, the exercising muscle blood flow increases in MH were smaller (~20%) than in TN; there was a decrease in blood flow to GIT, pancreas, spleen, pineal and pituitary glands in both conditions with this decrease greater in MH.

Conclusion

The combined loads of exercise and a hot environment required the same increases in muscle blood flow (as in TN) and greater heat dissipation (than in TN). Therefore, the smaller increases in muscle and skin blood flow together with the fall in CVP while MAP was maintained in MH, indicate that a) there is competition between metabolic and thermoregulatory requirements, and b) the demands for maintenance of MAP took precedence over both thermoregulation and muscle metabolism.
Furosemide results in an extracellular to intracellular fluid shift in horses

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Introduction

Furosemide (Lasix®) is a commonly used diuretic in horse racing and equine clinical practice. While pharmacology, pharmacodynamics, renal and hematological effects of furosemide have been studied in horses, its effects on the distribution of fluid within the horse remain unknown. The purpose was to describe the effects of furosemide on total body water (TBW) and the contributions from the extracellular and intracellular fluid compartments to the total renal fluid loss.

Material and methods

Horses were infused i.v. with 1 mg/kg body mass \( (n = 7) \) or 2 mg/kg \( (n = 9) \) furosemide. TBW, extracellular fluid volume (ECFV) and plasma volume (PV) were measured using D2O, NaSCN and Evans blue dilution. A change in ECFV was assessed from the change in plasma [protein] and from repeated infusion / dilution of NaSCN.

Results

Furosemide resulted in a 0.020 ± 0.002 L/kg decrease in TBW within 120 min. During the first 60 min, furosemide resulted in decreased ECFV and thereafter ECFV increased in 13 horses, remained unchanged in 2 and further decreased in 1. At 30 and 60 min after furosemide infusion, the decrease in ECFV determined by a change in plasma [protein] was 2-fold greater than when determined by repeated NaSCN infusion, with both determinations similar at 120 min. ECFV losses were nearly double the TBW losses, thus ECFV loss in excess of TBW loss is seen as an increase in ICFV.

Conclusion

Furosemide resulted in a net shift of fluid (electrolytes and water) from the extracellular to intracellular fluid compartment. This may be due to effects of the drug on skeletal muscle electrolyte cotransport systems, the KCl cotransporter or the Na-K-2Cl cotransporter. It is speculated that an increase in skeletal muscle cell volume may be linked to the performance enhancing effect of furosemide.
Does clenbuterol influence performance indices?

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Introduction

Clenbuterol (CLEN) is administered for its bronchodilator properties. Anecdotal reports suggest that CLEN may have performance enhancing properties, either through the described bronchodilator or other as yet unrecognized actions and thus becomes a regulatory issue. This study evaluated the effects of a CLEN treatment regime that provides quasi-steady circulating concentrations on various physiological indices associated with exercise performance.

Material and methods

Five Thoroughbred horses were used in a randomized crossover design that included 4 treatment groups; 2 placebo (CON) and 2 CLEN (1.6 ug/kg) oral treatments, with a 30-day clearance between administrations. Each treatment consisted of 13 administrations, every 12h. Horses exercised on a treadmill at speeds of 110% of at 1, 48, and 96h, or 24, 72, and 120h following the final treatment administration. Blood samples (arterial and mixed venous) were collected pre, 30 and 90 sec during and following exercise. Ventilatory parameters were measured using an open-flow mask system sealed to the horse's face; with an esophageal catheter used to measure pleural pressures.

Results

No significant differences were noted during exercise at any of the time points following CLEN administration including (mean ± SE, for e.g. pre-treatment and 1, 24h following the final CLEN administration: 175 ± 6, 171 ± 8, 172 ± 5 mL O2/min/kg, \( P = 0.17 \)), blood lactate concentrations (10.5 ± 1.2, 10.3 ± 0.9, 10.8 ± 1.4 mmol/L, \( P = 0.12 \)), peak inspiratory/expiratory esophageal pressures (32 ± 4/31 ± 8, 31 ± 6/30 ± 4, 34 ± 4/31 ± 6 mmHg, \( P = 0.22/0.19 \))

Conclusion

These data support the hypothesis that the airways of normal healthy horses are fully dilated during maximal exercise, and thus, treatment with bronchodilators will have no effect upon dynamic airway function.
Exercise-induced changes on lipid peroxides and antioxidant enzyme level changes in plasma of show jumping and dressage horses

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Introduction

Exercise is known to exert numerous physiological changes in the vital organ system of the body. Among those changes, the most important is the enhanced respiration and utilization of oxygen in the body. Increased oxygen influx during exhaustive exercise may be potentially harmful to the body. During recent years, much evidence has accumulated implicating an enormous generation of reactive oxygen species (ROS) and other free radicals specially during exercise in the muscles and hearth. Although the exact mechanism for the exercise-induced cell and tissue damage is still elusive, there is increasing evidence that the enhanced oxidative metabolism associated with the exercise can increase whole body oxygen consumption. Oxidative stress can be described as a disturbance in the antioxidant system which is not able to adequately scavenge free radical/reactive oxygen species and arrest lipid peroxidation. Cellular glutathione peroxidase (GPx) is a member of a family of enzymes whose function is to detoxify peroxides in the cell. Because peroxides can decompose to form highly reactive radicals, this enzyme plays a critical role in protecting the cell from free radical damage, particularly lipid peroxidation. Our main purpose was to investigate exercise-induced changes on GPx and lipid peroxide (LPO) plasma levels in show-jumping as well as dressage horses. Additionally, we also measured glutathione transferase (GST), catalase and glutathione plasma levels. For this purpose in this study we included 35 horses regularly involved in competition.

Material and methods

Blood samples were taken at three time points: baseline at rest, upon reaching the schooling area but before exercise and post-performance over a jump or dressage course. Eight healthy horses were considered as controls.

Results

Exercise induced an increase in LPO concentration in the two levels of horse jumping-show. We also observed an exercise-induced increase of LPO in the dressage horse, although the effect was less apparent. On the contrary, exercise decreased glutathione, GPx and GST levels.

Conclusion

In conclusion, exercise-induced oxidative stress is linked to an increased lipid peroxidation.
Effect of exercise on blood oxidant/antioxidant markers in standardbred horses: comparison between treadmill and race track tests

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Introduction

The present study aimed to compare in 6 healthy and trained standardbred horses the impact on the blood oxidant/antioxidant status of a standardised exercise test including a run up to fatigue performed on a treadmill (ETt, slope 6%) and on a racetrack (ETr).

Material and methods

The following blood antioxidant markers were analysed in jugular venous blood at rest (R) and 15 min after the test (E15): uric acid (UA), ascorbic acid (AA), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione (reduced: GSH and oxidised: GSSG), glutathione redox ratio (GRR) and derivative thiol in protein (PSH). Running time to fatigue (RTF), velocity during the last step (Vmax), final heart rate (HRfinal) and venous lactic acid (LA) were also determined during both tests.

Results

Vmax was significantly ($P < 0.05$) higher during the ETr (11.7 ± 0.25 vs. 11 ± 0 m.s⁻¹), whereas LA was significantly lower (10.95 ± 1.4 vs. 24.18 ± 0.52 mmol/L). HRfinal and RTF did not significantly differ between ETt and ETr. Exercise-induced significant increases (R vs. E15) of UA (8.2 ± 1.6 vs. 94 ± 11.3/µmol.L) and AA (16.4 ± 2.4 vs. 20.3 ± 3.5 µmol/L) were found in both tests, whereas GSH (1001 ± 37.1 vs. 859 ± 51 µmol/L) and PSH (324 ± 8.7 vs. 285 ± 18 µmol/L) significantly decreased. GPx, SOD, GSSG and GRR remained unchanged. Differences between ETt and ETr were significant at E15 for UA (159 ± 18.5 vs. 30 ± 4.1 µmol/L), GSH (837 ± 47 vs. 881 ± 56 µmol/L) and PSH (274 ± 19.8 vs. 297 ± 17 µmol/L).

Conclusion

For a same RTF and final HR, the treadmill test induced higher changes in blood lactate and in blood oxidant/antioxidant balance than did the race track test.
Effects of exercise on nitric oxide, cGMP, and cytokine levels in training horses

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Introduction

High intensity exercise induces oxidative stress and although there is not evidence that this affects sporting performance in the short term, it may have longer term health consequences. The mechanisms of exercise-induced oxidative stress are not well understood. Mitochondria are sometimes considered to be the main source of free radicals, but in vitro studies suggest they may play a more minor role than was first thought. An alternative mechanism by which exercise may promote free radical production involves ischaemia-reperfusion. Intense exercise is associated with transient hypoxia in several organs, as blood is shunted away to cover the increased blood supply in active skeletal muscles and the skin. Re-oxygenation of these tissues occurs after the cessation of exercise, and this can be associated with the production of ROS, as well as proinflammatory mediators. Furthermore, tissue damage and repair processes may involve the expression of inflammatory cytokines. Cytokines can act directly on target cells or they may stimulate the elaboration of secondary mediators such as other cytokines, arachidonic acid metabolites, cyclic nucleotides or free oxygen radicals. One of the molecular mediators that is increasingly implicated in cytokine action is nitric oxide (NO). The production of NO plays a vital role in the regulation of physiological processes and, both, proinflammatory as well as antiinflammatory effects have been described for this molecule. In this study we examined the effects of show-jumping as well as dressage on cytokines, nitric oxide and carbon monoxide levels by comparing horse exercise response at a horse show compared to their familiar home.

Material and methods

Blood samples were taken at three time points: baseline at rest, upon reaching the schooling area but before exercise and post-performance over a jump or dressage course.

Results

Exercise training induced an increase in NO levels in jumping horses, while it did not significantly modify NO levels in dressage horses. Exercise also increased CO levels in both jumping and dressage horses but this effect was more apparent in dressage horses. After exercise, plasma cGMP concentration was higher in both groups. Exercise also modifies cytokine profile.

Conclusion

Our results suggest that horses involved in competition can provide an excellent model to study the exercise-induced stress response. However, they show that the type of exercise applied induces different biochemical changes in plasma horses.
Influence of training on plasma adrenaline and noradrenaline kinetics in untrained Standardbreds

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Introduction

Catecholamines (CAT) play an important role in modulating the response to exercise. The kinetics of CAT changes during exercise are difficult to study due to their short biological half-life. Learning about variations in plasma CAT levels during training could furnish new information regarding sweating, redistribution of blood flow and energy metabolism.

Material and methods

Four untrained standardbred geldings aged 4-7 years, adapted to treadmill work, were used to determine the influence of training on plasma adrenaline (A) and noradrenaline (NA) kinetics. The horses underwent a standardized exercise test (SET) on treadmill before (SET1), 1 (SET2) and 2 months after (SET3) the start of a moderate training period on treadmill. The SET procedure was the following: warm-up and a single step of 2 min at 200 beats/min heart rate (SS). The automatic blood collection system (that has been carried out during SS) and the preparation of the horses have been described previously. The system was programmed to obtain a blood sample every 15s (8 blood samples per SS). The analyses were performed by HPLC. Training progress was monitored by means of anaerobic threshold (V4) and the speed during SS (V200) before and after the 2-month training. The Wilcoxon test was used for statistical analysis.

Results

V4 (m/s mean ± SD) changed from 7.3 ± 1.3 to 8.8 ± 1 and V200 from 8.4 ± 1.3 to 10.3 ± 1.3 after the training period. The results showed decreased levels of A and NA with significant differences between SET1/SET2 and SET1/SET3 for A (P = 0.007) and NA (P = 0.007). There were no differences between SET2 and SET3 for A (P = 0.195) and NA (P = 0.054).

Conclusion

These preliminary data seem to indicate that training influences the level of plasma CAT and that this influence is greater during the first training period in untrained standardbreds.
The effect of adrenergic suppression induced by guanabenz administration on exercising Thoroughbred horses

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Introduction
Guanabenz, an antihypertensive drug, depresses brain vasomotor and cardioaccelerator centers, peripherally blocks adrenergic neurons, and is reportedly used as a calming agent in horses. The objective of this study was to determine the effects of guanabenz-induced adrenergic suppression on fit Thoroughbred horses undergoing intense exercise.

Material and methods
In a random crossover design, twelve, conditioned Thoroughbred horses each received guanabenz (0.08 mg/kg IV) and placebo at a 3-week interval. An incremental exercise test to exhaustion on a treadmill followed treatment by one hour. Heart rate, oxygen consumption, carbon dioxide production, plasma lactate, catecholamines, ACTH and cortisol, and time to fatigue were monitored. Statistical analysis was performed using mixed-effects linear modeling.

Results
Mean heart rate during exercise was lower in guanabenz-treated horses (185.6 ± 2.4 vs. 189.1 ± 2.4 beats per minute, \( P = 0.0424 \)). Concentrations of plasma cortisol (138.4 ± 9.2 vs. 156.2 ± 9.0 ng/mL, \( P = 0.0183 \)) and epinephrine (1577.7 ± 294.0 vs. 2215.2 ± 285.4 pg/mL, \( P = 0.0289 \)) were lower for guanabenz-treated horses. Mean run time was slightly longer for guanabenz-treated horses, but did not obtain significance (232.8 ± 19.6 s vs. 226.7 ± 25.1 s, \( P = 0.0525 \)). No significant effects of guanabenz administration were found for oxygen consumption, carbon dioxide production, plasma lactate, norepinephrine or ACTH concentrations.

Conclusion
Guanabenz administration reduced plasma cortisol and epinephrine concentrations and heart rate, and may enhance endurance. Clear determination of a positive performance effect of epinephrine, but not norepinephrine suppression is needed before clinical significance can be determined.
Plasma b-endorphin, cortisol, and immune responses to acute exercise are altered by age and exercise training in horses

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Introduction

We hypothesized that training and age [young (Y ~7 yr.; n = 6), middle-aged (MA ~15 yr, n = 6), and old (O ~27 yr, n = 6)] would affect the plasma b-endorphin (BE) and cortisol (C) as well as immune function responses to acute exercise in unfit Standardbred mares.

Material and methods

A graded exercise test (GXT) was performed before and after 12 wks training at 60% HRmax. Leukocyte cell number, CD4+ and CD8+ cell subsets, mitogen stimulated lymphoproliferative response (LPR), and BE and C were measured in jugular blood. BE and C were measured at rest and at 5, 10, 20, 40, 60 and 120 min post-GXT.

Results

C rose by 5 min post-GXT in Y and MA mares (P < 0.05) and remained elevated until 40 min and 60 min post-GXT respectively during both pre- and post-training GXT. There was no rise in C in O mares post-GXT after either GXT (P > 0.05). Pre-training BE rose (P < 0.05) by 5 min post-GXT in all mares. After training, BE was higher in Y and O vs. MA (P < 0.05) at 5 min post-GXT. Post-training BE was higher at 5 min post-GXT in Y and O vs. pre-training (P < 0.05). BE was higher at 10 min post-GXT in O post-training vs. pre-training. Monocyte number was lower (P < 0.05) post-GXT in O vs. Y after training. After GXT, lymphocyte number rose in all mares (P < 0.05); however, lower lymphocyte numbers (P < 0.05) were seen in MA vs. Y and O vs. MA (P < 0.05). CD4+ lymphocytes were higher at rest in O and MA vs. Y (P < 0.05). A reduction (P < 0.05) in CD4+ lymphocytes was seen in O and MA after the pre-training GXT and in all mares after the post-training GXT. CD8+ lymphocytes rose (P < 0.05) after GXT in all ages. The O had reduced LPR to Con A stimulation (P < 0.05) compared to Y and MA after the GXT after both pre- and post-training GXT. Old mares displayed reduced (P < 0.05) LPR to PHA only after post-training GXT. LPR to PWM was lower (P < 0.05) in O vs. to Y and MA after the pre-training GXT. Training caused an increase in resting LPR to PWM in MA only (P < 0.05).

Conclusion

Both age and training alter the plasma b-endorphin and cortisol responses as well as immune responses to acute exercise.
Hormone response to training and competition in athletic horses

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Introduction

Exercise training is associated with peripheral-cellular and central-cerebral processes, hormonal-neuronal regulation and transmission mechanisms. During the acute training response, peripheral cellular mechanisms are mainly metabolistic to achieve energy supply and involve associated hormonal response. During overreaching and overtraining, a myopathy-like state is observed in skeletal muscle with depressed turnover of contractile proteins. These alterations are associated with increased cortisol and decreased insulin secretion and beta adrenergic stimulation. Previous studies examining the effects of exercise on adrenocortical function in humans have yielded conflicting results. Short-term exercise with light or moderate workloads may produce an increase, decrease or no change in plasma cortisol levels. The progressive rise in plasma cortisol associated with exercise is dependent upon the intensity and duration of the exertion as well as the fitness of the individual. It is recognized that the amount of psychological stress that an animal encounters determines the degree of response of the pituitary-adrenal axis. In athletes, the added competition is an important element in the adrenal response. However, the effect of competition on stress levels in equine athletes remains to be elucidated. In this study we examined the effects of show-jumping as well as dressage on stress levels by comparing horse stress response at a horse show compared to their familiar home.

Material and methods

Blood samples were taken at three time points: baseline at rest, upon reaching the schooling area but before exercise and post-performance over a jump or dressage course. Stress responses were assessed through changes in plasma adrenaline, cortisol and ACTH concentrations before and after exercise compared to at rest levels. In addition we also determine insulin, glucose and somatostatin and glucose plasma levels.

Results

Jumper horses displayed higher baseline cortisol concentrations compared with control horses whereas dressage horses showed lower baseline cortisol levels. Both, show-jumping as well as dressage horses showed lower basal ACTH plasma levels. In jumper horses, plasma cortisol levels did not differ from normal, at rest concentrations, upon reaching the schooling area, while they were elevated in dressage horses. The exercise resulted in a significant increase in both show-jumping as well as dressage horses, and this effect was more apparent in less experienced horses. ACTH levels were elevated after competition. Exercise also induced modifications in adrenaline, insulin and glucose plasma concentration.

Conclusion

This study demonstrated that horses do display a classic physiological stress response and that different training programs induce different responses.
Blood glucose and its possible relation with gender differences in the non-specific immune response to exercise

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Introduction

Elevated specific immune responses and the higher incidence of autoimmune diseases in female (compared to male) humans and animals have been known for a long time. These sex differences have been attributed to a multifactorial process including genetics, hormones, environmental factors and behaviour (Butts and Sternberg, 2004).

Material and methods

Little is known about the influence of gender on the non-specific immune system in exercise. This led us to consider this influence in horses after aerobic exercise (150 bpm). A group of 12 horses (6 males and 6 females) of between 4 and 6 years of age was studied observing their adherence properties (AI), chemotaxis, phagocytosis and superoxide production of neutrophils. In plasma, cortisol, catecholamines and glucose were analysed.

Results

ANOVA analysis determined significant differences in AI and glucose with the gender, both parameters being greater in the males (AI= 20.17 ± 1.66% and glucose= 5.55 ± 0.21 mmol/L) than in the females (AI= 14.17 ± 0.80% and glucose= 3.80 ± 0.14 mmol/L). A multiple regression analysis demonstrated a negative correlation between glucose and superoxide production in females.

Conclusion

It has been demonstrated that diabetes leads to an increased superoxide production of neutrophils (Yasunari et al, 2002), but it is not known what role blood glucose may play. The correlation observed in females (p=0.026) could indicate an improvement in the superoxide production of neutrophils with lower levels of blood glucose equalling the phagocytic effectiveness in both sexes in spite of the higher AI in males.
Changes in plasma glucose and insulin concentrations during standardised exercise tests and during experimentally induced early overtraining in Standardbreds

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Introduction

Twelve Standardbred geldings aged 1.5 years were selected for a longitudinal study to evaluate the changes in plasma glucose and insulin concentrations during exercise and experimentally induced overtraining.

Material and methods

Training was divided into four phases: 1) acclimatisation to exercise on the treadmill for 4 weeks; 2) training for 18 weeks by means of alternating endurance (~ 60% HRmax) and intensity exercise (~80% HRmax); 3) 6 weeks of increased training load, and 4) detraining for 4 weeks (~60% HRmax). In phase 3, horses were randomly divided into control and overtraining. Control horses exercised 4 days/week, whereas overtrained horses exercised 6 days/week during the first three weeks of phase 3 and 7 days/week with enhanced exercise load during the last three weeks. At the end of each phase, a standardised exercise test (SET) was performed at ~80% HRmax (8-8.5 m/s and 1-2.5% incline) for 20 minutes. Insulin was assessed by means of a validated radioimmunoassay. Statistical differences were assessed by the Huynh-Feldt and the Paired t-test. P values < 0.05 were considered significant. The results are presented as mean ± SD.

Results

Complete data sets were obtained from six horses. All three overtrained horses were unable to finish the third SET with normal handling in contrast with the control horses. Mean (n = 12) plasma glucose concentration decreased significantly 5 minutes after the start of SET 1 (from 4.53 ± 0.28 to 4.16 ± 0.34 mmol/L). Mean (n = 6) plasma glucose concentration was unchanged 5 minutes after ending SET 1 associated with a significant rise in mean plasma insulin (from 24.9 ± 12.7 to 66.0 ± 30.1 pmol/L).

Conclusion

Preliminary data showed a decrease in mean plasma glucose concentration during SET 1 and a trend suggesting early-overtraining to be parasympathetic in Standardbreds as reflected by lower plasma glucose concentration.
Blood viscosity changes during maximal exercise

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Introduction

The equine athlete has a unique ability to contract its spleen during exercise and dramatically increasing the volume of circulating red blood cells and blood hematocrit (Hct). These changes also increase whole blood viscosity (WBV) and alter blood rheology. Blood viscosity is a function of shear-rate, and changes in the vasculature of organs and vessel diameter will influence shear-rate. Generally, as flow velocity increases, viscosity decreases. This study describes the shear-viscosity profile of blood (a non-Newtonian fluid) as described by the Casson model in resting and exercising horses.

Material and methods

A unique ex-vivo scanning capillary viscometer system (Rheologies, Exton, PA) was used to measure WBV over a wide range of shear rates (1 to 1000 s⁻¹). This study reports changes in WBV in 6 TB horses prior, during and following exercise at 110% of the speed elicited. Results

WBV at rest, during 110% (30s and 90s), and during recovery (5, 15, 30, 60 min) at a shear-rate of 1s⁻¹ were the following: 12.5 ± 5.1, 36.0 ± 15.0, 57.3 ± 9.0, 47.4 ± 6.6, 37.9 ± 13.6, 24.5 ± 10.5, 15.2 ± 5.9 centipoises (cP), respectively. At a shear-rate of 1000s⁻¹ blood viscosity was 3.2 ± 0.50, 6.0 ± 1.6, 6.6 ± 0.87, 6.4 ± 1.7, 4.9 ± 0.88, 3.8 ± 0.54, 3.5 ± 0.49 cP, respectively. Hematocrit increased from 37.3 ± 2.6 to 59.3 ± 4.0 at rest and 90s, respectively. Percentage increases in the viscosity profile from rest to 90s exercise, at shear-rates of 1s⁻¹ and 1000s⁻¹ were 79.5 ± 6.0 and 51.3 ± 6.3, respectively. The equivalent % increase in Hct from rest to 90s was 36.9 ± 4.9.

Conclusion

Changes in the shape of the shear-viscosity profile indicate rheological changes beyond those attributed to increases in hematocrit. These changes are of particular interest when considering tissue perfusion alterations in the maximally exercising horse.
The effects of dietary n-3 enriched supplementation on erythrocyte membrane fatty acid composition and fluidity in exercising horses

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Introduction

The changes in membrane fluidity of erythrocytes may modify the erythrocyte function like rheological properties (deformability) and oxygen release. The aim of this study was to assess the effect of exercise with and without an oral antioxidant supplementation enriched with n-3 fatty acids on erythrocyte membrane fluidity (EMF) and erythrocyte fatty acid composition in eventing horses.

Material and methods

Twelve healthy and trained horses were randomly divided into two groups: group S received during 4 weeks an oral antioxidant cocktail enriched in n-3 fatty acid (alfa tocopherol, eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) whereas group P was placebo-treated. At the end of the treatment period, all horses performed a standardised exercise test (ET) under field conditions. Venous blood was sampled before starting the treatment (T0), immediately before (T1) as well as 15 min (T2) and 24 hours (T3) after ET. Spin labelled (16-DOXYL-stearic acid) red blood cell membranes were characterised using the relaxation correlation time (Tc in inverse proportion to EMF). Fatty acid composition (%) of the membranes was determined by gas-liquid chromatography.

Results

Supplementation did not induce changes in EMF (T1 vs. T0) but significantly increased erythrocyte n-3 polyunsaturated fatty acids (PUFA: C18.3n-3: + 10%, P < 0.05; C20.5n-3: + 124%, P < 0.05; C22.6n-3: + 311%, P < 0.05), n-3/n-6 ratio (+ 86%, P < 0.05), and total n-3 fatty acids (+ 64%, P < 0.05). An early exercise-induced decrease of EMF was noted in group P (T2 vs. T1: Tc: + 19%, P < 0.05), whereas a decrease of EMF in group S only occurred during the recovery period (T3 vs. T2: Tc: + 29%, P < 0.05). Erythrocyte membrane fatty acid composition did not change after exercise regardless of the group.

Conclusion

An oral antioxidant supplementation enriched with n-3 fatty acids induced changes in membrane composition, which might have modulated and delayed the exercise-induced decrease in EMF.
Hydration of exercised Standardbred racehorses assessed non-invasively using multi-frequency bioelectrical impedance analysis

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In human and animal clinical practice, multi-frequency bioelectrical impedance analysis (MF-BIA) is increasingly being used as a diagnostic tool to rapidly and non-invasively assess the hydration of the whole body as well as the intracellular and extracellular fluid compartments. Accurate determination of changes in hydration status within individuals over time has remained problematic due to the requirement for complete impedance-frequency relationships at the time points of interest. The purpose of this study was to use MF-BIA in 13 Standardbred racehorses and 7 endurance research horses to determine if MF-BIA could be used to track changes in total body water (TBW), intracellular fluid volume (ICFV) and extracellular fluid volume (ECFV) resulting from exercise. Jugular venous blood was sampled at rest and for 2 to 13 h following exercise. TBW, ECFV and plasma volume (PV) were measured at rest using indicator dilution techniques (D2O, thiocyanate and Evans Blue, respectively). TBW, ECFV, ICFV and PV were correlated to impedance measures and predictive equations were used to determine hydration status from MF-BIA measures. TBW loss continued throughout the recovery period, and was primarily borne by the ECF compartment at 90 min of recovery. MF-BIA predictions of compartmental hydration status were significantly correlated to measured / calculated decreases in these compartments.

<table>
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<th>Pre-Exercise measured</th>
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<th>BIA</th>
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<td>111*</td>
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<td>471</td>
<td>467</td>
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</tr>
</tbody>
</table>

*significantly less than pre-exercise (p<0.05); n = 20 horses.

Supported by Equistat Ltd., Isle of Man and the Natural Sciences and Engineering Research Council of Canada. Supported by Equistat Ltd., Isle of Man and the Natural Sciences and Engineering Research Council of Canada.
Is improved performance following furosemide administration due to diuresis-induced weight loss or reduced severity of exercise-induced pulmonary hemorrhage?

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Introduction

We hypothesized that improved performance after administration of furosemide is due to diuresis-induced weight loss rather than reduction in EIPH.

Material and methods

6 thoroughbred horses known to have mild EIPH underwent three standardized exercise tests (SET) in random order: control (C) in which horses received an iv saline placebo; furosemide/unburdened (FU), with horses receiving furosemide (0.5 mg/kg iv) 4 h before exercise; and furosemide/burdened (FB) in which horses received furosemide and were burdened with weight equal to that lost during the 4 h post-furosemide period. Bronchoalveolar lavages (BAL) were performed before and after each SET to assess the volume of EIPH. The SET involved 3 mins at 40% VO2max followed by galloping to fatigue at 115% VO2max. Erythrocyte numbers in BAL fluid and VO2max were recorded. Data were analyzed using a one-way repeated measures analysis of variance and a post-hoc Tukey test when the F statistic was significant (P < 0.06).

Results

VO2max (mL/kg.min) was higher (P < 0.001) for FU (152.9 ± 1.37) than for FB (148.6 ± 1.00) or C (148.3 ± 1.15). More RBC (x106/ml BAL fluid) were found after C (2.60 ± 0.71) than after FU (0.95 ± 0.12, P = 0.043) or FB (1.02 ± 0.14, P = 0.053).

Conclusion

FU and FB were associated with less evidence of EIPH than C, but VO2max was only higher with FU. This suggests that improved performance after furosemide administration is more due to diuresis and weight-loss than the drug’s apparent alleviation of EIPH. Working with horses that have severe EIPH may produce different results, and similar research with such horses is warranted.
Effects of a high and low daily salt (NaCl) intake

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Introduction

Horses on most diets need to be supplemented with NaCl. However, it is not known if a high daily NaCl intake will cause Na retention or how fast athletic horses develop Na deficiency on a non-supplemented diet. In the present study, athletic horses were offered a high and low NaCl diet for three weeks, respectively.

Material and methods

Four endurance horses were fed a high NaCl diet (H, supplemented with 0.18 g NaCl/kg BW) and a low NaCl diet (L, non supplemented) in a change-over design. At the end of each three week period, urine and faeces were collected for 48 h followed by a standardised exercise test (ET) (25.9 km, 1.6-4.8 m/s). Blood samples were taken at rest, during and after exercise.

Results

The urine production increased significantly ($P < 0.05$) with the H diet compared to the L diet (1.7 ± 0.04 vs. 1.3 ± 0.11 % of BW/day). The high Na intake was well regulated by an increased urinary and faecal Na excretion. With the L diet, plasma volume was reduced at rest and the plasma levels of aldosterone (pA) were elevated. Although pA levels were high following the ET on the L diet (3625 ± 613 vs. 279 ± 43 ñmol/L on H), the horses were not able to decrease faecal Na losses indicating that faecal Na excretion already was maximally reduced. All horses lost 11-15.5 kg of BW during the ET and 24h after the ET three horses had regained their pre-exercise BW on the H diet whereas only one horse had regained its body weight on the L diet.

Conclusion

Three weeks without NaCl supplementation was enough to reduce plasma volume and increase the recovery time after exercise. No signs of Na retention could be observed with a high daily Na intake.
Effects of sodium bicarbonate administration on performance indices

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Introduction

The ergogenic effects of sodium bicarbonate (NaHCO3) on athletic performance of horses have been debated. Anecdotal information suggests that NaHCO3 improves performance by increasing the buffering capacity of the blood and delaying the onset of acidity-induced fatigue. This study evaluated the effects of orally administered NaHCO3 on indices of performance during maximal treadmill exercise.

Material and methods

Five horses participated in three trials in a randomized crossover design; receiving placebo (CON) or NaHCO3 (0.3g/kg (LD) or 1.2g/kg (HD)) administered 4h prior to treadmill exercise of 2min at 110% of speed required to elicit. Blood samples (arterial, venous, and mixed venous) were collected pre, 30 and 90 sec during and following exercise. Ventilatory parameters were measured using an open-flow mask system sealed to the horse’s face. An esophageal catheter was used to measure pleural pressures. Biopsies from the middle gluteal muscle were collected prior to, immediately following maximal exercise and after 30 minutes of recovery.

Results

Following 90s maximal exercise, oxygen consumption was significantly reduced (mean ± SE: 175 ± 6.3, 177 ± 5, 170 ± 7 mL O2/min/kg, CON, LD, HD, respectively, \( P < 0.0001 \)) and blood lactate concentration significantly increased (10.4 ± 1.3, 10.2 ± 0.4, 13.6±1.9 mmol/L, CON, LD, HD, respectively, \( P = 0.02 \)). HD treatment also resulted in significant increases in venous base excess and pH (\( P < 0.001 \)). Accumulation of lactate in gluteal muscle tended to increase in a dose dependent manner (47 ± 11, 76 ± 3, 85 ± 22 umol/g dry muscle mass, CON, LD, HD, respectively \( P = 0.15 \)).

Conclusion

These data indicate that NaHCO3 administration results in reduced aerobic metabolism and increased anaerobic metabolism. This may negatively impact athletic performance.
Changes in arterial, mixed venous and intraerythrocytic concentrations of ions in supramaximally exercising horses

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Introduction
Horses experience major perturbations in acid-base balance during supramaximal exercise. The aim of this study was to clarify the role erythrocytes play in mitigating perturbations in acid-base balance during high speed exercise, and to describe associated differences in arterial (a) and mixed venous (v) concentrations of key ions.

Material and methods
6 fit horses galloped to fatigue at 115% VO2max. Blood samples were collected pre-exercise, at fatigue, and immediately post-exercise. Haematocrit (Hct), pH, PCO2, and plasma concentrations of bicarbonate (HCO3), chloride (Cl), sodium (Na), potassium (K), and lactate (La) were determined, and intraerythrocytic (i-rbc) concentrations of La and electrolytes calculated for each sample. Data were analysed using a 2-way ANOVA for repeated measures testing for effects of sampling time and site.

Results
Hct was 63 ± 1% at fatigue. Corresponding pH and PCO2 values were 7.12 ± 0.04 (a) and 6.99 ± 0.03 (v), and 57 ± 3 (a) and 136 ± 10 (v) Torr. Plasma and i-rbc [Cl] were increased with the hypercapnia and acidaemia. [HCO3]v was greater (30.1 ± 0.8 mmol/L) than pre-exercise values at fatigue, although [HCO3]a was lower (19.6 ± 1.0 mmol/L). Hyperkalaemia and decreased i-rbc [K] were evident at fatigue, as was an increased i-rbc [Na]. These changes reversed post-exercise. Concentrations of all measures of La rose from fatigue to post-exercise (i-rbca La = 10.2 ± 2.3 and 16.5 ± 2.9 mmol/L respectively). The strong ion difference decreased with exercise and was higher in v (41 ± 1.2 vs. 33 ± 1.5) at fatigue and post-exercise (31 ± 2.2 vs. 27 ± 1.9), reflecting the decrease in pH.

Conclusion
Erythrocytes act as a repository for lactate, and thus the increase in Hct facilitates the maintenance of the muscle to the plasma La diffusion gradient.
Effect of cross country on plasma levels of myeloperoxidase in saddle ponies

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Introduction

In humans, exercise has been shown to induce neutrophil degranulation, respiratory burst activity, as well as an increase in myeloperoxidase (MPO). To assess whether the same occurs in horses, degranulation of blood neutrophils after exercise was assessed by measuring the total concentration of MPO by ELISA, and the active fraction of MPO by SIEFED (Specific Immuno Extraction Followed by Enzymatic Detection) a new-patented method.

Material and methods

In nine ponies selected for the European Eventing Championship, blood was sampled before (T0), as well as 5 (T5) and 30 (T30) minutes after the cross phase. The cross length was 3340m, with 21 fences of 1.10 m (level FEI *). White blood cells (WBC) and percentage granulocytes, total plasma protein (TPP), creatine phosphokinase (CPK), as well as total and active MPO contents were determined at T0 and T30. Plasma lactate was measured at T5.

Results

The mean speed was 516 ± 40m/min. The mean blood lactate at T5 was 15.8 ± 5.8mmol/L (range:9.6 to 25.0mmol/L). Performing the cross country induced a significant decrease of the granulocytes, and a significant increase of CPK and TTP. MPO concentration was increased at T30 with a mean value of 388 ± 159ng/mL versus 159 ± 44ng/mL at T0 (P = 0.001) No significant correlation was found between MPO and the other parameters. Active MPO was also increased at T30 with a mean value of 3.44 ± 1.54mU/mL versus 2.23 ± 1.00 mU/mL at T0 (P < 0.05).

Conclusion

Intense exercise induced an activation of blood neutrophils, with degranulation and release of MPO, which remained active. The values of MPO after exercise approached the values observed in some inflammatory pathologic conditions. This phenomenon could partly contribute to exercise-induced oxidative stress.
Biochemical markers of bone activity in relation with the osteoarticular status in pre-training horses

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Introduction
All horse breeds are affected by developmental orthopaedic diseases resulting in substantial economic damages to the horse industry. Bone osteocalcin (BGP), hydroxyproline (HOP) and alkaline phosphatases (bone fraction or bone ALP) are markers of bone activity. Modifications of their concentrations in blood may result in disrupted bone growth inducing orthopaedic lesions.

Material and methods
The aim of this study was to determine the kinetics of these markers and to analyse their variations in relation with the osteoarticular status (OAS) in horses.
The growth of 674 horses of three breeds, Thoroughbreds, French Trotters and French Saddle horses, was followed from birth to 18 months. Jugular venous blood samples were taken regularly to measure BGP, HOP and ALP by normalised techniques.
The osteoarticular status was evaluated in the yearlings by radiography of the limbs. The severity of all the radiographic findings was considered to determine if the horse was normal without any lesion, moderately or severely affected.
The exponential model was used to determine the kinetics of BGP, HOP and ALP in each breed for the three groups of OAS.

Results
At birth, BGP and HOP concentrations were significantly higher in normal horses (respectively 190 ng/mL and 35 mg/L) than in horses with lesions (respectively 150 ng/mL and 24 mg/L). During the first months, BGP and HOP remained higher in severely affected horses. At 6 months, the mean values for BGP and HOP were 70 ng/mL and 7 mg/L respectively for all horses. The kinetics of PAL did not change with the OAS.

Conclusion
According to our results, the measure of BGP and HOP concentrations at birth and the following of their kinetics during the first months could be useful to detect early deficiencies in bone formation and to prevent further bone diseases such as osteochondrosis.
Efficacy of different methods for cooling horses after exercise under hot conditions

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Introduction

Horses generate considerable internal heat burdens when exercising. While it is common practice for a trainer or groom to place a wet blanket or towel on the dorsum of a hot horse post-exercise, there are no data supporting the efficacy of this cooling method. Newer breathable fabrics have been designed which, when pre-moistened, promote evaporative heat loss in human subjects while exercising or laboring (“cooling vests”). The objective of this experiment was to test the hypothesis that a pre-moistened cooling blanket designed with multilayered breathable material would enhance heat loss in horses post-exercise.

Material and methods

Eight treadmill-trained horses (7 TB, 1 QH, mean age 6.9 yrs) performed a standardized exercise test (SET) weekly for 3 weeks, with 3 different recovery treatments administered randomly. Pulmonary artery temperature (PAT) was measured via a Swan-Ganz catheter. The SET consisted of 10 min at 3.7 m/s, 3 min at 11.0 m/s, 25 min at 3.7 m/s, and 20 min of recovery walking at 1.8 m/s (58 min exercise and recovery under laboratory conditions of 36.7-40.6 C and 40-50% RH). From 3-6 min during recovery, the treadmill was stopped and the horses randomly received one of the following: (a) no bath (negative control), (b) a bath consisting of 32 L of 4 C water (positive control), or (c) application of a special fabric blanket soaked in 18 C water, wrung out, and placed over the horse’s dorsum and sides. PAT was compared using RM ANOVA with the Student Neuman-Keul test used post hoc to discriminate between treatments at specific points in time.

Results

Mean PAT rose with each phase of exercise ($P < 0.05$) and peaked at 40.2 ± 0.2 C. During recovery, the cold bath decreased PAT through 10 min after walking resumed ($P < 0.05$). The blanket did not decrease PAT compared to the negative control ($P > 0.05$), and both were hotter than the cold bath treatment through 16 min of recovery ($P < 0.05$).

Conclusion

A specially-designed cooling blanket failed to reduce PAT when compared to a negative control. Cold water bathing decreased PAT ($P < 0.05$) but was not effective throughout all of recovery when walking resumed after the bath.
Contribution of exercise intensity and duration to training-linked myosin transitions in Thoroughbreds

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Introduction

Although there is much literature describing muscular adaptations to training, the impact of relevant exercise parameters such as intensity and duration on this response remains to be clarified. This study defines the contribution of these factors on the myosin heavy chain (MHC) response to training in racehorses.

Material and methods

Six 2-3-yr-old Thoroughbreds performed, following a randomised 6x6 latin square design, 6 consecutive conditioning programmes of varying lactate-guided intensities (V2.5, V4) and durations (5, 15, 25 min). Each one, lasting 22 d, consisted of 11 exercise sessions once a d, every 2nd d) on a 6% inclined treadmill and followed by a 10 d resting period between consecutive programmes. Gluteus muscle biopsies (depth, 2 and 6 cm) were taken before and after each programme and electrophoretically analysed for MHC.

Results

Overall, training increased the fraction of MHC2A (mean ± SD, 41.1 ± 3.5% to 43.4 ± 4.42% \( P = 0.0004 \)) and decreased that of the MHC2X isoform (from 50.2 ± 4.4% to 47.4 ± 5.6% \( P = 0.001 \)). Exercise intensity fixed effects on fast MHC were highly significant \( (P < 0.01) \), whereas exercise duration only had a marginal effect \( (P = 0.0751) \) on the 2A:2X MHC ratio. On a per-individual basis, training impact on fast MHC was only significant \( (P < 0.05) \) in horses exercised for the longest duration at both intensities.

Conclusion

The short-term training-induced up-regulation of MHC2A and down-regulation of MHC2X in Thoroughbreds are more dependent on the intensity than the duration of the exercise. However, protocols with exercises of moderate intensity and long duration can induce MHC changes close to those promoted by exercise programmes of higher intensities.