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Endless thanks to all the individuals, labor and financial supporters, that have made the following 200 journal articles and book chapters possible.

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AGING AND HOW IT AFFECTS THE PHYSIOLOGICAL Response to Exercise in the Horse

K.H. McKEEVER

The use of older horses for athletic and/or recreational activities is a common practice, with many animals still actively competing or working even at ages greater than 20 years old. Research involving human athletes shows an age-related reduction in the ability to perform strenuous exercise; however, limited data are available regarding the exercise capacity of aged horses. Advancements in medical care as well as our understanding of nutritional and activity requirements of geriatric horses have led to many horses living well into their 20s and even 30s. Thus, there is an increased demand for a more thorough knowledge of the effects of aging on exercise ability. This review discusses the effects of aging on major physiological systems and the resulting impact on athletic performance.

Key Words: heart • *lungs* • *insulin-like growth factors* • *thermoregulation* • *immune response*



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AGE RELATED DECREASES IN THERMOREGULATION AND CARDIOVASCULAR FUNCTION IN HORSES

K.H. MCKEEVER, T.L. EATON, S. GEISER, C.F. KEARNS and R.A. LEHNHARD

Reason for performing study: Older horses have an increased risk of hyperthermia due to impaired cardiovascular function. While many studies have investigated thermoregulation in horses during exercise, none have investigated the effects of ageing.

Objective: To test the hypothesis that there is a difference in thermoregulation during exercise and plasma volume (PV) in young and old horses.

Methods: **Study 1:** 6 young (Y, 7.7 \pm 0.5 years) and 5 old (O, 26.0 \pm 0.8 years) unfit Standardbred mares (507 \pm 11 kg, mean \pm s.e.) ran on a treadmill (6% grade, velocity calculated to generate a work rate of 1625 watts) until core temperature reached 40°C. Core (CT), skin (ST), rectal temperature (RT) and heart rate (HR) were measured every min until 10 min post exertion. Packed cell volume (HCT), lactate (LA) and plasma protein (TP) were measured in blood samples collected before, at 40°C and every 5 min until 10 min post exercise. Sweat loss was estimated using bodyweight. **Study 2:** Plasma volume was measured in 26 young (8.2 \pm 0.7 years) and 8 old (26.6 \pm 0.7 years) Standardbred mares (515 \pm 12 kg) using Evans Blue dye. Pre-exercise blood (rBV) and red cell (rRCV) volumes were calculated using PV and HCT. Data analysis utilized repeated measures ANOVA and t tests and data are expressed as mean \pm s.e.

Results: Old horses reached 40°C faster (998 ± 113 vs. 1925 ± 259 s; P<0.05) with a greater HR at 40°C (184 ± 6 vs. 140 ± 5 beats/min; P<0.05) and greater sweat losses (P<0.05). Heart rate did not differ (P>0.05) post exercise. Age did not alter (P>0.05) CT, ST, RT, LA, HCT or TP. Plasma volume was greater in Y vs. O horses (P<0.05, 28.5 ± 1.4 vs. 24.1 ± 1.6 l) as was rBV (41.3 ± 2.0 vs. 35.3 ± 2.3 l) and rRCV (13.3 ± 0.6 vs. 11.1 ± 0.8 l).

Conclusion: Ageing compromises the ability to handle the combined demand of exercise and thermoregulation in part due to decreased absolute pre-exercise.

Key Words: horse • *exercise* • *thermoregulation* • *cardiovascular* • *plasma volume*



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THE EFFECT OF AGE AND EXERCISE TRAINING ON INSULIN SENSITIVITY, FAT AND MUSCLE TISSUE CYTOKINE PROFILES AND BODY COMPOSITION OF OLD AND YOUNG STANDARDBRED MARES

N.R. LIBURT, M.N. FUGARO, K. MALINOWSKI, E.K. WUNDERLICH, J.L. ZAMBITO, D.W. HOROHOV, A. BETANCOURT, R.C. BOSTON, R.J. GEOR, J. ONISHI and K.H. MCKEEVER

This study tested the hypothesis that old and young mares exhibit different endocrine responses to a frequently sampled intravenous glucose tolerance test (FSIGT) and different cytokine profiles in blood, adipose, and muscle tissues. It was also hypothesized that exercise training alters endocrine and tissue cytokine profiles. Pilot data from 15 mixed background horses indicated tissue differences in cytokine profiles. For the main study, six old (22.0 \pm 0.7 years) and six young (7.3 \pm 0.6 years; mean \pm SE) unfit Standardbred mares were tested pre- and post-training. Exercise training occurred three days/week for 15 weeks at ~60% maximum heart rate. Plasma insulin and glucose concentrations were measured via radioimmunoassay and enzyme-electrode interface, respectively. Samples of blood, middle gluteal muscle (RM), and subcutaneous adipose tissue from the neck (NF) were collected pre- and post-training for mRNA quantification. Minimal model analysis of FSIGT, repeated measures ANOVA and Pearson Product Moment were used to analyze data. The null hypothesis was rejected when $P \leq 0.10$. Posttraining, old and young mares improved insulin sensitivity (SI) (P=0.08, P=0.01, respectively) and disposition index (P=0.04, P<0.001, respectively), but acute insulin response to glucose increased in young mares only (P=0.02). Old mares exhibited lower (P=0.06) average relative quantity (RQ) of tumor necrosis factor-alpha in NF compared to RM. Old mares showed greater RQ of interleukin-6 (IL-6) in NF compared to young (P=0.08). A negative correlation was observed between SI and IL-6 in NF post-training (P=0.07, R=-0.54), and a positive correlation occurred between SI and monocyte chemotactic protein-1 in NF post-training (P=0.08, R=0.53). Exercise training improved pancreatic beta cell function and insulin sensitivity in old and young horses. Cytokines associated with glucose metabolism may have varied roles in different tissues.

> *Key Words: cytokine* • *conditioning* • *glucose* • *horse* • *endocrine*



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EFFECT OF EXERCISE ON THE IMMUNE RESPONSE OF YOUNG AND OLD HORSES

N.R. LIBURT, M.N. FUGARO, K. MALINOWSKI, E.K. WUNDERLICH, J.L. ZAMBITO, D.W. HOROHOV, A. BETANCOURT, R.C. BOSTON, R.J. GEOR, J. ONISHI and K.H. MCKEEVER

Objective: To compare exercise-induced immune modulation in young and older horses.

Animals: 6 young and 6 aged horses that were vaccinated against equine influenza virus.

Procedure: Venous blood samples were collected for immunologic assessment before and immediately after exercise at targeted heart rates and after exercise for determination of plasma lactate and cortisol concentrations. Mononuclear cells were assayed for lymphoproliferative responses and incubated with inerleukin-2 (IL-2) to induce lymphokine-activated killer (LAK) cells. Antibodies to equine influenza virus were measured.

Results: Older horses had significantly lower proliferative responses to mitogens than younger horses prior to exercise. Exercise caused a significant decrease in lymphoproliferative response of younger horses, but not of older horses. Activity of LAK cells increased slightly with exercise intensity in younger horses. Cortisol concentrations increased in both groups after exercise; younger horses had higher concentrations after exercise at heart rates of 180 and 200 beats/min than those of older horses. Plasma lactate concentrations increased with exercise intensity but there were no differences between older and younger horses. Older horses had lower antibody titers to equine influenza virus than younger horses. Exercise did not affect antibody titers.

Conclusion: Although lymphoproliferative responses and antibody titers of older horses were less than those of younger horses, older horses were more resistant to exercise-induced changes in immune function, possibly because of lower cortisol concentrations.

Clinical Relevance: Stress and aging are known to affect immune function. Older horses had reduced immune function, but were more resistant to exercise-induced immune suppression than younger horses.

Key Words: stress • aging • immune system • exercise



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EFFECT OF TRAINING ON AGE-RELATED CHANGES IN Plasma insulin and glucose

K. MALINOWSKI, C. L. BETROS, L. FLORA, C. F. KEARNS and K. H. MCKEEVER

The purpose of the study was to determine whether 12 weeks of exercise training would affect plasma glucose and plasma insulin concentrations in young and older Standardbred mares. Eighteen healthy, unfit mares representing 3 age groups were used: young (Y = 6.8 ± 0.4 years; n = 6), middle-aged (MA = 15.2 ± 0.4 ; n = 6), and old (O = 27.0 ± 0.2 ; n = 6). Pre- and post-training incremental exercise tests (GXT) were performed to measure plasma glucose and insulin concentration from immediately after, until 120 min post-exercise. Training consisted of exercise 3 days/week (weeks 1-8) and 4 days/week (weeks 9–12) at a submaximal intensity (~60% of pre-training HR_{max}) for ~30 min/day. Old mares had lower levels of glucose after the GXT when compared to Y and MA mares (P<0.05). There was also a time-by-age interaction (P = 0.003) in that, at 120 min post-GXT, glucose levels had not returned to pre-exercise values. Plasma glucose concentrations, in response to acute exercise, were not altered by training for any age group (P>0.05). Prior to exercise training, O mares had higher plasma insulin compared to Y and MA mares at 120 min post-exercise (P<0.05), and insulin was also elevated in O mares compared to all other time points at 120 min post-GXT (P<0.05). Training resulted in increased plasma insulin concentrations at 120 min post-GXT in all age groups (P<0.05). In conclusion, age affected glucose and insulin responses to acute exercise prior to and after 12 weeks of exercise training. With regards to insulin, 12 weeks of exercise training resulted in a post-exercise rebound hyperinsulinaemia, which may be related to an increased need for glycogen repletion in the muscle. These factors are important in considering the ability of an older horse to tolerate exercise.

> Key Words: horse • insulin • glucose • training • ageing



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EFFECTS OF AGE AND RECOMBINANT EQUINE Somatotropin (eST) administration on immune function in female horses

D.W. HOROHOV, A.N. DIMOCK, P.D. GUIRNALDA, R. FOLSOM, K. MALINOWSKI and K.H. MCKEEVER

Aging has been associated with declines in somatotropin and IGF-1 levels as well as declines in immune function. To determine the effects of age and whether ST administration could reverse immunosenescence in horses, eight young and eight aged female Standardbred horses were given 10mg/ recombinant equine somatotropin (eST) or vehicle buffer for 49 d. Plasma IGF-1 concentrations in both age groups were higher in eST-treated animals (P<0.001), and higher in young eST-treated mares than in aged eSTtreated mares during wk 4 to 7 (P<0.001). There was a trend toward lower monocyte and granulocyte numbers (P=0.07) in mares treated with eST. Aged mares treated with eST had lower lymphocyte numbers (P<0.005). The percentage of CD4⁻ lymphocytes was higher in aged mares (P<0.001), and the percentage of CD8⁺ lymphocytes was higher in young mares (P<0.01). Lymphocyte proliferation in response to concanavalin A, phytohemagglutinin, and pokeweed mitogen was not lower in aged mares (P = 0.17, 0.17, and 0.13 respectively). Aged mares treated with eST showed a lower peak primary antibody response to keyhole limpet hemocyanin (P<0.05). Young mares treated with eST showed a higher peak primary antibody response to keyhole limpet hemocyanin (P<0.05). Like other species, horses exhibit similar signs of age-related declines in various immune parameters, but those of aging were not reversed with eST treatment.

Key Words: aging • *horses* • *insulin-like growth factor* • *senescence* • *somatotropin*



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EFFECTS OF AGEING AND TRAINING ON MAXIMAL HEART RATE AND VO_{2max}

C. L. BETROS, K. H. MCKEEVER, C. F. KEARNS and K. MALINOWSKI

The purpose of this study was to test the hypotheses that ageing would result in a decline in maximal heart rate (HR_{max}) and maximal aerobic capacity (VO_{2max}) and, secondarily, that those effects would be reversible with training. Eighteen, healthy, unfit Standardbred mares representing 3 age groups: young (Y = mean \pm s.e. 6.8 \pm 0.4 years, n = 6); middleaged (MA = 15.2 ± 0.4 years, n = 6); and old (O = 27.0 ± 0.2 years, n = 6) were used. HR_{max}, VO_{2max} and oxygen pulse at VO_{2max} (OP_{max}) and the velocities producing HR_{max} (VHR_{max}) and VO_{2max} (VVO_{2max}) were measured during pre-training and post-training incremental exercise tests (GXT). During training, mares exercised 3 days/week (Weeks 1–8) and 4 days/week (Weeks 9–12) at a submaximal intensity (~60% HR_{max}) for ~30 min/day. There were no differences (P>0.05) between Y and MA, before (218 \pm 2 vs. 213 \pm 3 beats/min; 116 \pm 3 vs. 109 \pm 3 ml/kg bwt/min; 0.55 \pm 0.01 vs. 0.52 \pm 0.02 ml/kg/beat; 9.0 ± 0.3 vs. 9.3 ± 0.2 m/s; 8.8 ± 0.2 vs. 8.8 ± 0.2 m/s) or after training (224 ± 2 vs. 218 ± 2 beats/min; 131 ± 3 vs. 120 ± 2 ml/kg bwt/min; 0.58 ± 0.01 vs. 0.55 ± 0.01 ml/kg/beat; 10.5 \pm 0.2 vs. 9.5 \pm 0.1 m/s; 10.6 \pm 0.2 vs. 9.5 \pm 0.1 m/s) for HR_max, VO_2max, OP_max, VHR_max or VVO_{2max}, respectively. Old horses had lower HR_{max}, VO_{2max} and OP_{max} and reached them at lower velocities compared to Y and MA (P<0.05), both before $(193 \pm 3 \text{ beats/min}; 83.2)$ \pm 2.0 ml/kg bwt/min; 0.43 \pm 0.01 ml/kg/beat; 7.8 \pm 0.1 m/s; 7.2 \pm 0.1 m/s) and after training $(198 \pm 2 \text{ beats/min}; 95 \pm 2 \text{ ml/kg bwt/min}; 0.48 \pm 0.01 \text{ ml/kg/beat}; 8.2 \pm 0.2 \text{ m/s};$ 8.0 ± 0.2 m/s). Training did not alter HR_{max} in any age group (P>0.05) but did cause increases in VO_{2max}, OP_{max} and VVO_{2max} for all groups (P<0.05). Interestingly, training increased VHR_{max} only in Y (P<0.05). These data demonstrate that there is a reduction in HR_{max} , VO_{2max} , OP_{max} , VHR_{max} and VVO_{2max} in old horses, and that training can partially reverse some effects of ageing.

> *Key Words: horse* • *equine* • *exercise* • *cardiovascular*

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EFFECT OF TRAINING ON INTRINSIC AND RESTING HEART RATE AND PLASMA VOLUME IN YOUNG AND OLD HORSES

C.L. BETROS, N.M. MCKEEVER, K. MALINOWSKI, H.C. MANSO, and K.H. McKEEVER

The chronic bradycardia seen in several species after intense exercise training may be due to autonomic mechanisms, non-autonomic mechanisms, such as increased pre-load, or a combination of the two. Thirteen, heathy, unfit Standardbred mares were split into two groups: young (age 12 ± 1 yr; mean \pm standard error, n=8) and old (age 22 ± 1 yr, n=5) to test the hypothesis that there would be age and training related differences in resting heart rate (RHR), intrinsic heart rate (IHR), maximal heart rate (HR_{max}) and plasma volume (PV). Mares were trained 3d/wk at 60% HR_{max} for 20 min and gradually increased to exercising 5d/wk at 70% HR_{max} for 30 min and RHR, IHR, HR_{max}, and PV were measured prior to and after the 8 wk training period. There were no age related differences (P>0.05) between young and old mares before $(41 \pm 2 \text{ vs. } 42 \pm 2 \text{ beats per}$ minute (bpm); 86 ± 5 vs. 80 ± 4 bpm) or after training (35 ± 1 vs 34 ± 1 bpm; 81 ± 6 vs. 78± 2 bpm) for RHR and IHR respectively. RHR was decreased (P<0.05) following training in both the young $(41 \pm 2 \text{ vs. } 35 \pm 1 \text{ bpm})$ and old mares $(42 \pm 2 \text{ vs. } 34 \pm 2 \text{ bpm})$. Training decreased IHR (P<0.05) in the young mares (86 ± 5 vs. 81 ± 6 bpm), but not (P>0.05) the old mares (80 ± 4 vs. 78 ± 2 bpm). The young horses had a higher HR_{max} than the old horses (P<0.05) both before (216 ± 5 vs. 200 ± 4 bpm) and after training (218 ± 3 vs. 197 \pm 5 bpm). Maximal heart rate was not altered after training (P>0.05) in either young (216 \pm 5 vs. 218 \pm 3 bpm) or old (200 \pm 4 vs. 197 \pm 5 bpm) mares. The PV of the young mares was 15% higher before training and 32% higher after training when compared to the old mares (P<0.05). Training caused an increase in PV in young mares (+9%; P<0.05), but did not alter PV in old mares (-5%; P>0.05). Training improved RHR in the young but no the old horses. The decrease in measured parameters in the young horses appears to be related to enhanced pre-load associated with a training=induced hypervolemia as well as changes in autonomic function.

> *Key Words: horse* • *autonomic nervous system* • *aging*



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ENDOCRINE RESPONSE TO EXERCISE IN YOUNG AND OLD HORSES

K.H. MCKEEVER and K. MALINOWSKI

Six young (mean + s.e., 5.3 ± 0.8 years, 445 ± 13 kg bwt) and 6 old (22.0 ± 0.4 years, 473 ± 13 kg bwt) 18 kg bwt) Standardbred and Thoroughbred mares were used to test the hypothesis that age would alter the endocrine response to exercise. All of the mares were unconditioned but accustomed to the laboratory, to standing quietly and running on a treadmill, and to the standardized incremental exercise test (SET) used in the experiment. Two weeks prior to the experiment, each horse underwent a SET to determine maximal oxygen uptake (VO_{2max}) and the speeds to be used in the actual experiment. A second graded exercise test (GXT) was performed without instrumentation for the measurement of plasma renin activity (PRA) and the plasma concentrations of atrial natriuretic peptide (ANP), arginine vasopressin (AVP), aldosterone (ALDO), and endotheli-1 (ET-1). Blood samples (30 ml) were collected at rest and at the end of each one-minute step of the exercise test. Plasma concentrations of hormones were measured using radioimmunoassay kits. There were no differences (P>0.05) between old vs. young mares for resting PRA (2.2 ± 0.3 vs. 1.5 ± 0.3 ng/ml/h), or the plasma concentrations of ANP (10.0 ± 0.9 vs. 10.7 ± 0.6 pg/ml); AVP (0.7 ± 0.7 vs. 1.4 ± 0.4 pg/ml); ALDO (39.2 ± 10.3 vs. 22.7 ± 4.6 pg/ml); or ET-1 (0.23 ± 0.04 vs. 0.18 ± 0.03 pg/ml). Exercise significantly increased PRA and the concentrations of ANP, AVP, and ALDO in both groups of horses; however, ET-1 was not altered (P>0.05) by exercise in either group. There were differences (P<0.05) between means obtained from the old and young groups for PRA (5.4 ± 0.6 vs. 3.9 ± 0.8 ng/ml/h) and the concentrations of ANP (14.5 ± 2.3 vs. 26.5 ± 9.0 pg/ml), AVP (13.6 ± 0.3 vs. 26.1 ± 13.9 pg/ml), and ALDO (76.8 \pm 22.0 vs. 41.5 \pm 4.9 pg/ml) measured in samples obtained at the speed eliciting VO_{2max}. These data suggest that older horses have an age-altered endocrine response to exercise.

> *Key Words: horse* • *exercise* • *aging* • *hormones*

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THE INTERSECTION OF INFLAMMATION, INSULIN RESISTANCE AND AGEING: IMPLICATIONS FOR THE STUDY OF MOLECULAR SIGNALING PATHWAYS IN HORSES

R.C. AVENATTI

Inflammation-associated insulin resistance contributes to chronic disease in humans and other long-lived species, such as horses. Insulin resistance arises due to an imbalance among molecular signaling mediators in response to pro-inflammatory cytokines in the aged and obese. The mammalian heat shock protein response has received much attention as an avenue for attenuating inflammatory mediator signaling and for contributing to preservation and restoration of insulin signaling in metabolically important tissues. Data on heat shock proteins and inflammatory signaling mediators in untrained and aged horses are lacking, and horses represent an untapped resource for studying the mediator imbalance contributing to insulin resistance in a comparative model.

> *Key Words: cytokine* • *diabetes equine* • *insulin* • *heat shock proteins*



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MAXIMAL AEROBIC CAPACITY (VO_{2max}) in horses: A retrospective study to identify the Age-related decline

A. WALKER, S.M. ARENT AND K.H. MCKEEVER

Previous studies of the effect of age on maximal aerobic capacity (maximal rate of oxygen consumption, VO_{2max}) in horses have only grouped horses (young, middle-aged and old) for statistical analysis. Those experiments were not designed to determine a break point due to age. The purpose of this study was to utilize data collected over the last 15 years to test the nature of the association between age and VO2max, and secondarily, to determine whether there is a 'breakpoint' in the age-related decline in aerobic capacity. Data were acquired from 50 unfit Standardbred mares ranging in age from 4 to 29 years, and were used in a retrospective regression analysis in order to characterize the agerelated decline in VO_{2max}. All VO_{2max} values were measured using the same incremental graded exercise test protocol. The data were analyzed using a curvilinear regression analysis predicting VO_{2max} from age, and the break point was determined using univariate simple contrasts. There was a significant decline in VO_{2max} with age (R²=0.554; P<0.001). The data analysis demonstrated a notable downturn in cardiopulmonary function between the ages of 18 and 20 years (P<0.001). Such results indicate similar responses to age between humans and horses. These results have implications for improving training methods in active older horses.

> *Key Words: equine* • *ageing* • *maximal aerobic capacity*



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MYOSIN HEAVY CHAIN PROFILES AND BODY COMPOSITION ARE DIFFERENT IN OLD VERSUS YOUNG STANDARDBRED MARES

R.A. LEHNHARD, K.H. MCKEEVER, C.F. KEARNS AND M.D. BEEKLEY

There are limited data on age-related changes in body composition or skeletal muscle in the horse. Therefore, the purpose of this study was to investigate any differences in muscle myosin heavy chain (MHC) and body composition associated with aging. Twenty-three young (4–8 years) and eight old (20+ years) unfit Standardbred mares were evaluated. Rump fat thickness was measured using B-mode ultrasound and percent body fat (% fat) was calculated. Needle muscle biopsies were obtained from right gluteus medius muscle. MHC composition was determined via sodium dodecyl sulphatepolyacrylamide gel electrophoresis. Three MHC isoforms were subsequently identified as type I, type IIA, and type IIX and quantified using a scanning and densometric system. There were no significant differences (P>0.05) between old and young mares in fat (%) $(19.0 \pm 6.4 \text{ vs } 20.5 \pm 5.4)$, fat mass (kg) $(102.3 \pm 39.9 \text{ vs } 106.9 \pm 37.1)$, or body weight (kg) $(529.4 \pm 34.9 \text{ vs} 512.7 \pm 57.7)$. However, the old mares had significantly (P<0.05) greater lean body mass than the young mares (427.1 \pm 24.5 vs 405.7 \pm 37.9). Aged mares had significantly (P<0:05) less type I ($7.8 \pm 2.9\%$ vs $12.1 \pm 4.4\%$) and IIA ($27.8 \pm 7.1\%$ vs 36.1 \pm 9.5%) fibres than the young group but more type IIX (64.6 \pm 4.7% vs 51.8 \pm 11.1%). The MHC data are consistent with the age-related changes seen in other species.

> *Key Words: horse* • *aging, muscle fibre* • *fat mass* • *fat-free mass*



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RESPONSE OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS TO STIMULATION TESTS BEFORE AND AFTER EXERCISE TRAINING IN OLD AND YOUNG STANDARDBRED MARES

N.R. LIBURT, K.H. MCKEEVER, K. MALINOWSKI, D.N. SMARSH AND R.J. GEOR

This study tested the hypotheses that age-induced alteration in cortisol, ACTH, and glucose concentrations are due to differences in the response of the hypothalamic-pituitary-adrenal axis and that exercise training would attenuate these differences. Six old (22.0 \pm 0.7 yr; mean \pm SE) and 6 young (7.3 \pm 0.6 yr) unfit Standardbred mares ran 3 graded exercise tests (GXT): before (GXT1), after 8 wk of training (GXT2), and at study end at 15 wk (GXT3). Mares trained 3 d/wk at 60% maximum heart rate. Each mare underwent 5 endocrine stimulation tests pre- and post-training: 1) control (CON), 2) adrenocorticotropin hormone (ACTH test), 3) combined dexamethasone suppression/ACTH (DEX/ACTH), 4) dexamethasone suppression (DEX), and 5) combined DEX/corticotropin releasing factor (DEX/CRF). For CON, there was no difference in plasma cortisol between age groups pre-training (P=0.19), but young mares had a 102% higher mean (P=0.02) plasma cortisol concentration than old mares post-training. The pre-training ACTH test showed young mares had a 72% higher (P=0.05) overall plasma cortisol concentration compared to old. There was no overall age difference in cortisol in the post-training ACTH test, but old mares still had lower cortisol concentrations at 30 min during the test, suggesting decreased adrenal response to ACTH stimulation. There was no difference in cortisol response between old and young mares in DEX, DEX/ACTH, or DEX/CRF tests. Young mares had higher (P=0.02) overall plasma cortisol concentration post-training in response to DEX/ACTH, but old mares showed no change. In CON and DEX/CRF, there were no age differences in plasma ACTH concentration, pre- or post-training. Pre-training, there was no age difference in glucose response to DEX, but post-training old mares had a 4% (P=0.04) lower overall plasma glucose concentration compared to young. Post-training, old mares had lower mean plasma glucose concentrations during DEX compared to pre-training (P=0.02), but there was no change prevs. post-training in young mares (P=0.19). Old and young mares had lower plasma glucose concentrations post-training during DEX/ACTH (P<0.001 and P=0.05, respectively) and DEX/CRF (P<0.001 and P=0.003, respectively) compared to pre-training. Both the pituitary and adrenal glands experience a decline in function with age although the exact mechanisms behind such changes remain unknown. Exercise training facilitates the counteraction of these deficits.

Key Words:

adrenocorticotropic hormone • age • cortisol • exercise • horse • hypothalamic-pituitary-adrenal axis



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A MODIFIED EVANS BLUE DYE METHOD FOR Determining plasma volume in the horse

K.H. McKEEVER, W.A. SCHURG and V.A. CONVERTINO

Six mature previously inactive horses were utilized for this study. Plasma volume determinations were made under controlled conditions on 2 separate occasions, 7 days apart. A previously published extraction and elution technique was adapted to process the plasma samples containing the Evans Blue dye. Doses of the dye were adapted for use in the horse. The major advantage of this procedure is that it separates the dye from its albumin carrier and removes substances that can interfere with spectrophotometric analysis, causing significant variation. Plasma samples can be run immediately or they can be frozen for several months after collection. There were no significant differences between the mean plasma volumes determined on Day 1 or Day 7. Mean plasma volume for the six horses was 15.7 + 0.7 liters for the first determination and 16.3 + 0.9 liters for the second. Average coefficient of variation for the method was 2.6%. In addition to being highly reproducible, this methodology does not require the use of radioisotopes. Finally, the extraction method allows for the use of lower doses of Evans Blue (20 mi of 0.5% aqueous solution) and the dye has a relatively short half-life which allows for repeated measurements of plasma volume.



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RETROSPECTIVE STUDY OF PREDICTIVE VARIABLES FOR MAXIMAL HEART RATE (HR_{max}) in horses undergoing Strenuous treadmill exercise

T.L. VINCENT, J.R. NEWTON, C.M. DEATON, S.H. FRANKLIN, T. BIDDICK, K.H. McKEEVER, P. MCDONOUGH, L.E. YOUNG, D.R. HODGSON and D.J. MARLIN

Reasons for performing study: Heart rate is one of the most commonly measured variables in equine exercise physiology and relative exercise intensity commonly expressed as % of maximal heart rate. A number of influences affect maximal heart rate (HR_{max}), including age of the horse but other factors have not been described.

Objectives: to determine if fitness, health status, gender breed, athletic use, body mass, in addition to age, are predictive of HR_{max} in the horse.

Methods: Maximal heart rate data from 328 horses which underwent treadmill exercise tests at 5 different laboratories were obtained retrospectively. Univariable linear regression analyses were performed on individual variables. Multiple linear regression analysis using a backward elimination modelling procedure was then used to relate the observed HR_{max} values simultaneously with different predictive variables. Variables were retained in the final regression model if they or any of their categories were significant predictive of HR_{max} at P<0.05 and f there was a significant collective contribution to the model from inclusion of each variable, also at P<0.05.

Results: Age, fitness status, laboratory, gender, and breed/use (combined category) were all statistically significantly predictive of HR_{max} , together these variables accounted for 41% of the variance in HR_{max} . Age alone accounted for only ~13% of the variation between horses in HR_{max} . Neither body mass nor health status were significantly predictive.

Conclusions: HR_{max} in the horse declines with age but is also influenced by other factors. As the factors investigated accounted for only 41% of the variation between horses, other unidentified variables with a strong influence on HR_{max} remain to be identified.

Potential relevance: Factors such as fitness, age, gender, breed and use need to be considered when interpreting estimates or measurements of HR_{max}.

Key Words: horse • Thoroughbred • Standardbred • cardiac • incremental exercise test • fatigue



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ACUTE PROGRESSIVE RESISTANCE EXERCISE ALTERS INTRACARDIAC PRESSURE IN PONIES

K.H. McKEEVER, S.E. ALWAY, J.W. FARRIS, K.W. HINCHCLIFF and J.A. LOMBARDO

Five male ponies $(3-6 \text{ years}, 208 \pm 11 \text{ kg})$ were used to examine the haemodynamic effects of resistance exercise. The experiment consisted of a 30 min catheterization period and a 15 min equilibration period followed by the test. Initially, the ponies stood quietly on a treadmill for collection of 5 min of baseline data. They then walked at 1.9ms⁻¹ for 1 min sets while carrying 0 kg, 67 kg or 134 kg of weight loaded on the saddle area. Weights were applied in a randomized order using a chain hoist and sling. The ponies rested unloaded for 2 min between sets and all cardiovascular parameters returned to baseline before beginning the next set. Haemodynamic measurements (heart rate (HR), carotid artery systolic (SP), diastolic (DP), pulse (PP) and mean pressure (MAP), right atrial pressure (RAP), right ventricular pressure (RVP) and right ventricular maximum rate of pressure rise (dP/dt_{max}) were recorded continuously and data collected during the last 18 s of each set were averaged and analyzed. HR increased (P<0.05) with unloaded exercise (201%) and with increases in load (252 and 271% at 67 and 134 kg, respectively). RAP increased (P<0.05) with exercise, with differences (P<0.05) between the response to the 0 kg (66%), 67 kg (121%) and 134 kg (138%) loads. RVP increased (P<0.05) incrementally with load during exercise (58, 110 and 136%, respectively), with differences (P<0.05) between 0 versus 67 kg and 67 versus 134 kg. Right ventricular dP/dt_{max} increased (P<0.05) incrementally and showed differences (P<0.05) between loads (131, 191 and 252% at 0, 67 and 134 kg, respectively). There were increases (P<0.05) in MAP (20%), SP (20%) and DP (27) with exercise, but no difference (P>0.05) between the response to 67 versus 134 kg load (43 and 51%; 40 and 46%; 49 and 62, respectively). Pulse pressure did not change (P>0.05) from baseline values. These data suggest that resistance exercise in the form of weight carried increases intraventricular pressure through a significant increase in both cardiac pre-load and after-load.

> *Key Words: heart* • *equine* • *weight-lifting* • *blood pressure* • *haemodynamics*



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APOPTOSIS AND ANTIOXIDANT STATUS ARE INFLUENCED BY AGE AND EXERCISE TRAINING IN HORSES

C.A. WILLIAMS, M.E. GORDON, C.L. BETROS and K.H. McKEEVER

Eight mature $(12 \pm 2 \text{ yr}; \text{MAT})$ and 5 older $(22 \pm 2 \text{ yr}; \text{OLD})$ Standardbred mares were used to test the hypothesis that aging and exercise training would alter apoptosis in white blood cells and antioxidant status. The horses were housed indoors overnight (16 h/d) in 3 m x 3 m stalls and were turned out in a drylot during the day. They were fed a diet consisting of total mixed ration, hay cubes fed ad libitum or an equine senior diet plus grass hay. Horses were trained for 20 to 30 min/d, 3 to 5 d/wk for 8 wk at a submaximal work intensity between 60 to 70% of maximal heart rate. A graded exercise test (GXT; stepwise test until exhaustion) was performed before (GXT1) and after (GXT2) the 8 wk of training. During the GXT, blood samples and heart rate were taken at rest, 6 m/s, fatigue, and at 5 and 60 min post-fatigue. Fatigue plasma lactate concentration was greater in MAT (19.3 \pm 1.5 at 10 m/s) compared with the OLD (10.9 \pm 1.2 mmol/L at 9 m/s; P=0.008) horses. There was no effect of age or training on plasma lipid hydroperoxide (LPO) concentration. However, there was a positive correlation between LPO and plasma lactate concentration (r=0.27, P=0.006) during acute exercise. There was a greater concentration of total glutathione after GXT1 than after GXT2 (111.8 \pm 5.0 vs. 98.6 \pm 3.4 μ M, respectively; P=0.0002) for both age groups. Apoptosis was less (P=0.002) in white blood cells of the MAT vs. the OLD group. These results demonstrate that older horses are under similar amounts of oxidative stress, measured by LPO, and have similar levels of glutathione in their systems compared with mature horses. The observation that more glutathione was needed during GXT1 for both groups of horses indicates that training helps horses adapt their system for the intense post-training exercise tests. The greater level of white blood cell apoptosis also indicates that older horses may be immune-compromised during exercise. However, research still needs to be performed regarding dietary supplementation in the aged horse.

Key Words: aging • *antioxidant* • *apoptosis* • *equine* • *exercise training* • *glutathione*



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ARTERIAL-VENOUS DIFFERENCE IN ATRIAL NATRIURETIC PEPTIDE CONCENTRATION DURING EXERCISE IN HORSES

K.H McKEEVER, K.W. HINCHCLIFF, J.L.COOLEY, D.R. LAMB AND W.W. MUIR III

Six nontrained mares were subjected to steady-state, submaximal treadmill exercise to examine the effect of exercise on the plasma concentration of atrial natriuretic peptide (ANP) in arterial, compared with mixed venous, blood. Horses ran on a treadmill up a 6 degree grade for 20 minutes at a speed calculated to require a power equivalent to 80% of maximal oxygen uptake (VO_{2MAX}). Arterial and mixed venous blood samples were collected simultaneously from the carotid and pulmonary arteries of horses at rest and at 10 and 20 minutes of exercise. Plasma was stored at -80° C and was later thawed; ANP was extracted, and its concentration was determined by radioimmunoassay. Exercise caused significant (P < 0.05) increases in arterial and venous plasma ANP concentrations. Mean \pm SEM arterial ANP concentration increased from 25.2 \pm 4.4 pg/ml at rest to 52.7 \pm 5.2 pg/ml at 10 minutes of exercise and 62.5 ± 5.2 pg/ml at 20 minutes of exercise. Mean venous ANP concentration increased from 24.8 ± 4.3 pg/ml at rest to 67.2 ± 14.5 pg/ml at 10 minutes of exercise and 65.3 ± 13.5 pg/ml at 20 minutes of exercise. Significant differences were not evident between arterial or mixed venous ANP concentration at rest or during exercise, indicating that ANP either is not metabolized in the lungs or is released from the left atrium at a rate matching that of pulmonary metabolism.



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ATRIAL NATRIURETIC PEPTIDE DURING EXERCISE IN HORSES

K.H. McKEEVER, K.W. HINCHCLIFF, L.M. SCHMALL, D.R. LAMB and W.W. MUIR III

Six unfit mares were subjected to maximal and steady state submaximal treadmill exercise to examine exercise-induced changes in the plasma concentration of atrial natriuretic peptide (ANP), a hormone with profound vasodilatory and renal effects, that is released by atrial stretch. In Experiment 1, ANP was measured at each step of an incremental maximal heart rate (HR) test. Exercise was started at 4 m/s, and speed was increased 1 m/s each min until HR reached a plateau. In Experiment 2, mares were randomly assigned to either an exercise (EX) or parallel control (CON) trial on day 1 and that alternate trial 1 week later. The horses ran on a treadmill, up a 6° slope, for 1 hour at 55-60% of HR_{max}. Central venous blood was collected at 0, 20, 40, and 60 min during EX or CON. Plasma was stored at -80° C and later thawed, extracted with C18 columns and assayed for ANP by a RIA kit (Peninsula Laboratories, Inc.) Plasma ANP increased 600% (P < 0.05) from 9 ± 1 pg/ml (mean ± SE) at rest to 63 ± 14 pg/ml at HR_{max} in Experiment 1, and from 11 ± 1 pg/ml at rest to a peak of 40 ± 9 pg/ml (264%, P < 0.05) at 40 min of EX in Experiment 2. During CON, ANP did not change (P > 0.05) from 13 ± 2 pg/ml at 0 min. there were no significant differences among the three baseline values, observed for the two experiments. Increases in ANP concentration were highly correlated with %HR_{max} (r = 0.92). These results suggest a potential role for increasing concentrations of ANP in the cardiovascular/renal response to maximal and submaximal exercise in horses.

> *Key Words: atrial natriuretic peptide* • *exertion* • *horses*



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CHANGES IN COAGULATION AND FIBRINOLYSIS IN HORSES DURING EXERCISE

K.H. McKEEVER, K.W. HINCHCLIFF, G. KOCIBA, REED, S.M. and W. MUIR

Changes in clotting time (CT) and fibrinolytic activity (FA) were evaluated in 6 mature, female horses during exercise. Two trials were performed on consecutive days, using a randomized crossover design. Each mare was assigned to either an exercise trial or a control trial on the first day, and to the alternate trial 24 hours later. Mares exercised for 20 minutes on a treadmill at an elevation of 2° and a velocity of 5m/s. Venous blood samples were collected immediately before exercise, at 4, 8, 12, 16, and 20 minute during exercise, and 15 minutes after cessation of exercise. Blood was placed into plain glass tubes for determination of CT, and into chilled, citrated tubes for determination of FA, plasminogen/plasmin complex activity (PLG), one-stage prothrombin time (OSPT), activated partial thromboplastin time (APTT), and antithrombin-III (AT-III) activity. There were significant differences (P<0.05) between the control and exercise groups for CT, FA, and PLG. During exercise, clotting time decreased from 21.5 ± 1.6 minutes to 9.9 \pm 1.6 minutes (mean \pm SD; P<0.05), without significant changes in OSPT, APTT, or AT-III. Fibrinolytic activity and PLG increased (P<0.05) during exercise. Changes in CT, FA, and PLG were significant at 4 minutes of exercise, remained altered until the end of exercise, and returned to baseline values by 15 minutes of recovery. Clotting time, OSPT, APTT, FA, AT-III, and PLG did not change (P>0.05) during control trials.



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COMPARISON OF OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN ENDURANCE HORSES IN THREE 80-KM RACES

C.A. WILLIAMS, D.S. KRONFELD, T.M. HESS, K.E. SAKER, J.E. WALDRON, K.M. CRANDELL AND P.A. HARRIS

This study tested our hypothesis that during an 80-km Research Ride in 2002 (R2) horses that did not finish (NF) the ride would have elevated muscle enzyme activities in the blood and changes in biomarkers of oxidative stress as compared to horses that finished (F) the ride. These results were then compared to previous rides – Old Dominion (OD) and the Research Ride 2001 (R1). For R2, 40 mostly Arabian horses competed and had blood samples collected before, at 27, 48 and 80 km, and 170 to 190 min after the 80-km race. Blood was collected similarly in R1 and OD. Blood was analyzed for plasma lipid hydroperoxides (LPO), α-tocopherol (TOC), creatine kinase (CK), aspartate aminotransferase (AST), red and white blood cell total glutathione (GSH-T) and glutathione peroxidase (GPx). Data were analyzed using a repeated measure ANOVA in SAS. Associations between muscle enzymes and antioxidant status were determined using Pearson's or Spearman's correlations. Activities of CK and AST were higher (P<0.05) before, during and after the ride in NF than in F; however, TOC, LPO, GSH-T and GPx were not different. In R2, negative correlations were found with GPx and CK (r=-0.21; P=0.005), GPx and AST (r=-0.15; P=0.05), and a positive correlation was found with GSH-T and CK (r=0.18; P=0.02). Values of CK, LPO, GPx and GSH-T were higher (P<0.05) in R2 than in R1 or OD. The overall comparison of 80-km endurance races suggests the importance of considering the horse's fitness, terrain, ambient conditions and calibre of race when interpreting results from markers of oxidative stress and muscle enzyme leakage.

Key Words:

antioxidant • *endurance* • *equine* • *muscle enzymes* • *oxidative stress*



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DETERMINANTS OF OXYGEN DELIVERY AND HEMOGLOBIN SATURATION DURING INCREMENTAL EXERCISE IN HORSES

C.K. FENGER, K.H. McKEEVER, K.W. HINCHCLIFF and C.W. KOHN

Objective: To determine components of the increase in oxygen consumption (VO₂) and evaluate determinants of hemoglobin saturation (SO₂) during incremental treadmill exercise in unfit horses.

Animals: 7 unfit adult mares.

Procedures: Horses performed 1 preliminary exercise test (EXT) and 2 experimental EXT. Arterial and mixed venous blood samples and hemodynamic measurements were taken during the last 30 seconds of each step of the GXT to measure P_{02} , hemoglobin concentration ([Hb]), S_{02} , and determinants of acid-base state (protein, electrolytes, and P_{CO2}).

Results: Increased VO₂ during exercise was facilitated by significant increases in cardiac output (CO), [Hb], and widening of the arteriovenous difference in O₂. Arterial and venous pH, Pa_{O2}, and P_{VO2} decreased during exercise. Arterial P_{CO2}, bicarbonate ([HCO₃⁻)], decreased significantly, whereas P_{VCO2} increased. Arterial and venous sodium concentration, potassium concentration, strong ion difference, and venous lactate concentration all increased significantly during exercise.

Conclusions and Clinical Relevance: Increases in CO, [Hb], and O₂ extraction contributed equally to increased VO₂ during exercise. Higher P_{CO2} did not provide an independent contribution to shift in the oxyhemoglobin dissociation curve (ODC) in venous blood. However, lower Pa_{CO2} shifted the curve leftward, facilitating O₂ loading. The shift of ODC resulted in minimal effect on O₂ extraction because of convergence of the ODC at lower values of P_{O2}. Decreased pH appeared responsible for the rightward shift of the ODC, which may be necessary to allow maximal O₂ extraction at high blood flows achieved during exercise.



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EFFECT OF EXERCISE INTENSITY ON PLASMA PROSTAGLANDIN CONCENTRATIONS IN HORSES

L.A. MITTEN, K.W. HINCHCLIFF, J.L. PATE, C.W. KOHN and K.H. McKEEVER

Exertion has an effect on plasma, serum, and/or urine prostanoid concentrations in many species. We investigated the effect of exercise intensity on plasma prostaglandin concentrations during and after exercise in horses. Six Thoroughbreds completed 4 trials; 3 exercise trials (low-, medium-, and high-speed) and 1 non-exercise (control) trial on a high-speed treadmill. Blood samples were collected from a jugular catheter before, during, and after exercise. The PCV and blood lactate, plasma protein, plasma prostacyclin (6-keto-PGF_{1a}), thromboxane (TXB₂), and prostaglandin E₂ (PGE₂) concentrations were measured before, during, and after exercise is significantly (P=0.001) increased plasma TXB₂ concentrations during and after exercise in the low-, medium-, and high-speed trials, although effect of exercise intensity was not detected. Exercise was associated with an increase in PCV and blood lactate and plasma protein concentrations. There was no effect of exercise on plasma 6-keto-PGF_{1a} concentrations; PGE₂ was not detected in plasma from any horse in any trial.



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EFFECT OF AMBIENT TEMPERATURE AND HUMIDITY ON PULMONARY ARTERY TEMPERATURE OF EXERCISING HORSES

C.W. KOHN, K.W. HINCHCLIFF and K.H. McKEEVER

Six healthy Thoroughbred mares were trained to run on a high-speed treadmill and were conditioned for approximately 5 weeks. Each horse performed 6 identical standardized exercise tests (SET) 5 to 7 days apart. Each SET was performed under different ambient laboratory conditions: low temperature/low humidity (LL) $20.2^{\circ} \pm 0.6^{\circ}$ C, $53.6 \pm 4.1\%$; low temperature/high humidity (LH) $19.7^{\circ} \pm 0.6^{\circ}$ C, $86.7 \pm 4.2\%$; medium temperature/low humidity (ML) $24.6^{\circ} \pm 0.2^{\circ}$ C, $58.7 \pm 3.7\%$; medium temperature/high humidity (MH) $24.7^{\circ} \pm 0.3^{\circ}$ C, $87.5 \pm 1.4^{\circ}$; high temperature/low humidity (HL) $31.1^{\circ} \pm 0.6^{\circ}$ C, $41.6 \pm 1.4^{\circ}$ 3.5%; and high temperature/high humidity (HH) $30.6^{\circ} \pm 0.2^{\circ}$ C, $84.4 \pm 3.6\%$. Horses completed the 46 min SET, became fatigued or exercise was terminated when a pulmonary artery temperature (PAT) of 41.5°C was reached. During a 30 min recovery period, horses stood quietly on the treadmill. Only one trial was terminated due to fatigue; in all other trials horses completed the 46 min SET or the trial was terminated when PAT = 41.5°C. The relationships among run time (min) or PAT (°C), ambient temperature and % relative humidity were defined by multiple linear regression analysis. Run time (min) = 90.9 - (1.39 x ambient temperature $^{\circ}$ C) - (0.236 x relative humidity %). Approximately 54% of the variation in run times was explained by variation in environmental conditions, indicating that ambient temperature and humidity contribute substantially to the rate of rise in PAT. Run times to a PAT of 41.5°C were significantly shorter (P<0.000001) in HH than in other groups during the SET. Approximately 63% of the variation in PAT at the end of exercise was due to ambient temperature and humidity. In general, the longer the exercise time, the better the correlation among PAT, ambient temperature and relative humidity. Rectal temperature increased significantly (P<0.000001) during the recovery period only in horses in the HH group, indicating that heat dissipation during recovery may have been impaired by environmental conditions. This study demonstrates that hot humid conditions result in more rapid rate of rise in PAT for horses undertaking identical exercise tests.

> *Key Words: horse* • *thermoregulation* • *cooling* • *fatigue*



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EFFECTS OF HIGH ALTITUDE AND EXERCISE ON PLASMA ERYTHROPOIETIN IN EQUIDS

K.H. McKEEVER, S.J. WICKLER, T.R. SMITH and D.C. POOLE

To help resolve the mechanistic bases for haematological adaptations (~28% increase in red blood cell volume) of equids to high altitude (3800 m, barometric pressure Pb, 487 mmHg) and exercise, plasma erythropoietin concentration ([EPO]) was measured at rest and following exercise in six, moderately fit equids (four Arabians, one Quarter Horse and one Shetland Pony; four females and two males; age 9.0 ± 4.5 years (mean \pm SD)). [EPO] was measured on 2 days at 225m (i.e. ~sea level; P_b, 743 mmHg), over the course of a 10-day altitude exposure, and then again for 2 days after return to sea level. A standard track exercise test (submaximal, speed set-to-heart rate of 110 (trot), 150 (canter), 180 (gallop) bpm) was performed 2 days pre-high-altitude exposure and on three separate days at high altitude. In addition, a maximal incremental exercise test was performed on a high-speed motor-driven treadmill at sea level and 2 days following return to sea level from high altitude. Resting [EPO] increased from 28 ± 29 at sea level to $144 \pm 46 \text{ mUml}^{-1}$ (P<0.05) on the first day at high altitude. By day 2 at high altitude, [EPO] had returned to baseline $(31 \pm 24 \text{ mUml}^{-1}, \text{P}>0.05 \text{ vs. pre-high altitude})$ and did not change over the remaining 8 days at high altitude nor over the 2 days after return to sea level. [EPO] was not significantly altered by acute exercise at sea level or at 3800 m. These results indicate that [EPO] increases rapidly (though transiently) in response to hypobaric hypoxia but not to acute exercise, and that exercise does not appear to potentiate the altitude response. Thus, if any [EPO]-derived haematological adaptations to high altitude are present, these appear to result from a transient ~4-fold elevation of [EPO] rather than any sustained increase in this signalling mechanism, at least in the equid.

> *Key Words: equine* • *exertion* • *hypoxia* • *high altitude*



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EFFECTS OF SHOW-JUMPING PERFORMANCE STRESS ON PLASMA CORTISOL AND LACTATE CONCENTRATIONS AND HEART RATE AND BEHAVIOR IN HORSES

M.E. COVALESKY, C.R. RUSSONIELLO and K. MALINOWSKI

Effects of show jumping competition on stress levels in horses was examined. Twenty-six horses were sampled in both show and farm environments from three levels of show experience schooling, (least experience, n=11); intermediate, (moderate experience, n=6); open, (most experience, n=9). Blood samples were taken at 6 time points; 3 at the horseshow and 3 at the home farm (baseline at rest, pre-schooling and post-performance over a jump course). Stress responses were assessed through comparison of the behavioral response of the horses (determined by a subjective scoring system) to plasma cortisol (F) concentrations. Physical exertion was evaluated by heart rate (HR) and plasma lactate (L) concentrations. Schooling jumpers displayed higher baseline F concentrations than open jumpers (83.5 ± 9.2 vs. 46.4 ± 10.2 ng/ml, respectively; P<0.05); but not when compared to intermediate jumpers (66.2 ± 13.7 ng/ml; P>0.05) at the horse show.

At the farm, there were no differences in F concentrations between levels at a time point (P<0.05). Least experienced horses displayed higher F values at rest at the show compared to the home farm. At the show, open jumpers had higher L concentrations post-exercise compared to intermediate horses ($40.3 \pm 3.4 \text{ vs}$. $25.5 \pm 4.0 \text{ mg/dL}$, respectively; P<0.05); but were not different compared to schooling jumpers ($34.8 \pm 2.0 \text{ mg/dL}$; P>0.05). There were no differences in heart rates or behavior score between experience levels at specific time points at either the show or farm (P>0.05). Low positive correlations were found between HR and F (r=0.24, P<0.05); HR and L (r=0.48, P<0.05); and HR and behavior (r=0.22, P<0.05). Results suggest that conditioned jumpers that have previously been exposed to horse show environments do not appear stressed during acute show-jumping competition.



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THE EFFECT OF TRAINING AND CESSATION OF TRAINING ON PLASMA TOTAL CARBON DIOXIDE IN STANDARDBRED HORSES

R.A. LEHNHARD, A.M. SZUCSIK, V. BALASKONIS, K. MALINOWSKI and K.H McKEEVER

Twenty-seven mature Standardbred mares (9–27 years, ~522 kg) were used to test the hypothesis that training and short-term cessation of training would alter total plasma total carbon dioxide (tCO2) concentrations. Plasma tCO2 concentrations were measured in blood samples (20 ml) collected at rest from the same ten unfit mares that were used in two separate studies that were conducted 1 month apart. Comparisons between the samples obtained from the two trials were made to demonstrate the consistency of plasma tCO2 concentrations in the untrained population. Another set of resting blood samples was collected from an additional 17 mares that were being utilized in a training study that was underway during the same period. All the mares were housed in groups on dry lots, and were fed approximately 12 kg of mixed alfalfa-grass hay divided into two feedings per 24 h period. During the 'detraining' period, the 17 horses were housed in 5 x 5 m stalls, and were fed the same hay ration. Water and trace-mineral blocks were available ad *libitum.* Blood samples were collected from the mares prior to the last session of their 12 weeks of training at 60% HR_{max} (maximum heart rate), as well as on the third day following 2 days of detraining (simulated quarantine). Plasma tCO2 concentrations were measured in duplicate using a Beckman EL-ISE analyser. For data analysis, *t*-tests with the *a priori* level of statistical significance set at P<0.05 were used. Resting plasma tCO2 concentrations were lower (P<0.05) in the trained horses $(31.4 \pm 1.9 \text{ mMol } l^{-1})$ than in the untrained horses $(34.4 \pm 0.9 \text{ mMol } l^{-1})$. There were no effects (P>0.05) due to cessation of training. It was concluded that training causes a decrease in plasma tCO2 concentrations that is not reversed by limited cessation of training.

> *Key Words: plasma tCO2 concentration* • *training* • *detraining*



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ENDOTHELIN RESPONSE DURING AND AFTER EXERCISE IN HORSES

K.H. McKEEVER, C.F. KEARNS and L.A. ANTAS

The objective of the present study was to measure plasma endothelin-1 (ET-1) at rest and during exercise in the horse. Six healthy, Standardbred and Thoroughbred mares (5.3 ± 0.8 years; 445.2 ± 13.1 kg) which were unfit, but otherwise accustomed to running on the treadmill, were used in the study. Plasma ET-1 concentrations were measured using a commercially available radioimmunoassay kit. Horses performed three trials: a standing control (CON) trial where blood was collected from the jugular vein every minute for 5 min; a graded exercise test (GXT) where blood samples were collected at the end of each 1 min step of an incremental exercise test; and a 15 min submaximal (60% VO_{2max}) steady-state exercise test (SST) where blood samples were collected 1 min before, immediately after and at 2 min, 10 min, and 20 min post-exercise.

Plasma ET-1 concentration did not change (P>0.05) during the CON trial where it averaged 0.18 ± 0.03 pg/ml (mean \pm SE). Surprisingly, plasma ET-1 concentration did not change during the GXT trial where it averaged 0.20 ± 0.03 pg/ml. There were no differences between the mean concentrations obtained in either trial (P>0.05). Plasma ET-1 concentrations were, however, significantly elevated (P<0.05) immediately following exercise and at 2 min post-exercise in the SST. Post-exercise plasma ET-1 concentrations returned to baseline (P>0.05) by 10 min of recovery. Together, these data may suggest that ET-1 concentrations are altered in response to an exercise challenge.

> *Key Words: equine* • *cardiovascular* • *endocrine*



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EXERCISE CAPACITY IN YOUNG AND OLD MARES

K.H. McKEEVER and K. MALINOWSKI

Objective: To test the hypothesis that, compared with unfit young horses, unfit older horses have lower aerobic capacity and reduction in other indices of exercise capacity.

Animals: 6 young (mean \pm SEM, 5.3 \pm 0.8 years and 445 \pm 13 kg) and 6 aged (22.0 \pm 0.4 years and 473 \pm 18 kg) healthy Standardbred and Thoroughbred mares.

Procedures: The mares, accustomed to running on a treadmill, were tested by use of an incremental exercise test. None of the mares had received exercise training for at least 4 months prior to the study. During testing, mares ran up a fixed 6% grade starting at a speed of 4 m/s with 1 m/s increase every 60 seconds (omitting 5 m/s) until they reached fatigue. Maximal oxygen uptake (VO_{2max}) was measured by use of an open-flow calorimeter. Venous blood samples (10 ml) were collected during the last 10 seconds of each step and were used to measure blood lactate concentration and PCV. Calculated performance indices included velocity at VO_{2max}, maximal velocity, and velocity at lactate concentration of 4 mmol/L; work rate (watts) at those velocities also was determined.

Results: There were differences (P<0.05) between old and young mares for maximal run velocity attained during the test (8.7 ± 0.5 vs. 10.8 ± 0.5 m/s, respectively), VO_{2max} (89.4 ± 4.3 vs. 117.3 ± 9.5 ml/kg of body weight/ml, respectively), and velocity at VO_{2max} (8.0 ± 0.4 vs. 9.8 ± 0.7 m/s, respectively). Also, velocity required to reach blood lactate concentration of 4 mmol/L was lower (P<0.05) in old (7.5 ± 0.4 m/s), compared with young (10.2 ± 0.7 m/s), mares.

Conclusion: Older mares have substantially (-24%) lower maximal aerobic capacity than do young mares.

Clinical Relevance: Many horses participate in athletic activities into their late teens and some do so beyond the age of 20 years; thus, the need exists to explore ways to adjust training programs for older horses.



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EXERCISE-INDUCED ALTERATIONS IN PLASMA CONCENTRATIONS OF GHRELIN, ADIPONECTIN, LEPTIN, GLUCOSE, INSULIN, AND CORTISOL IN HORSES

M.E. GORDON, K.H. McKEEVER, C.L. BETROS and H.C. MANSO FILHO

Six Standardbred (STB) mares (11 ± 2 years, 521 ± 77 kg; means \pm SD) performed an exercise trial (EX) where they underwent an incremental exercise test (GXT) as well as a parallel control trial (CON) to test the hypothesis that short-term, high intensity exercise would alter plasma concentrations of glucose, leptin, adiponectin, ghrelin, insulin and cortisol. Plasma samples were taken before (0 min), during (last 10 s at 6, 8 m/s, and the velocity eliciting VO_{2max}), and after exercise (2, 10, 30, 60 min; 12 and 24 h post-GXT). A second set of blood samples was collected before and after an afternoon meal given at 1515 h (at 1500, 1514, 1530, and 1545 h). Data were analyzed using ANOVA for repeated measures and Tukey's test. During the GXT, there were no changes (P >0.05) in the plasma concentrations of glucose, leptin, adiponectin or ghrelin. However, there was a 29% increase (P<0.05) in mean plasma cortisol concentration and a 35% decrease (P<0.05) in mean plasma insulin concentration. Substantial increases (P<0.05) in the mean plasma concentrations of glucose and cortisol of 36% and 102%, respectively, were seen in the EX trial during the first 60 min post-GXT. Plasma leptin concentration, measured at the 24 h post-GXT time point, was 20% lower (P<0.05) during the EX trial compared with the parallel time point in the standing control (CON) trial. Plasma ghrelin concentration was 37% lower (P<0.05) in the EX trial compared with CON before and after the afternoon meal, but was 43% higher (P<0.05) 12 h post-GXT. There were no differences between EX and CON for plasma concentrations of insulin or adiponectin during recovery. It was concluded that short-term high intensity exercise alters plasma leptin and ghrelin concentrations in STB mares post-exercise, which may signal the exercised animals to alter energy intake.

> *Key Words:* exercise, • ghrelin • leptin • adiponectin • horse



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EXERCISE-INDUCED INCREASES IN INFLAMMATORY CYTOKINES IN MUSCLE AND BLOOD OF HORSES

N.R. LIBURT, A.A. ADAMS, A. BETANCOURT, D.W. HOROHO and K.H. McKEEVER

Reasons for performing study: Studies have demonstrated increases in mRNA expression for inflammatory cytokines following exercise in horses and have suggested those markers of inflammation may play a role in delayed onset muscle soreness. However, measurement of mRNA expression in white blood cells is an indirect method. No studies to date have documented the cytokine response to exercise directly in muscle in horses.

Hypothesis: This study tested the hypothesis that exercise increases cytokine markers of inflammation in blood and muscle.

Methods: Blood and muscle biopsies were obtained from 4 healthy, unfit Standardbred mares (~500 kg). The randomised crossover experiment was performed with the investigators performing the analysis blind to the treatment. Each horse underwent either incremental exercise test (GXT) or standing parallel control with the trials performed one month apart. During the GXT horses ran on a treadmill (1 m/s increases each min until fatigue, 6% grade). Blood and muscle biopsies were obtained 30 min before exercise, immediately after exercise and at 0.5, 1, 2, 6 and 24 h post GXT or at matched time points during the parallel control trials. Samples were analysed using real time-PCR for measurement of mRNA expression of interferon-gamma (IFN-gamma), tumour necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6) and interleukin-1 (IL-1). Data were analysed using t tests with the null hypothesis rejected when P<0.10.

Results: There were no changes (P>0.10) in IL-1, IL-6, IFNgamma or TNF-alpha during control. Exercise induced significant increases in IFN-gamma, IL1 and TNF-alpha in blood and significant increases in IFN-gamma, IL-6 and TNFalpha in muscle. There were no significant changes in mRNA expression of IL-1 in muscle or IL-6 in blood following the GXT. These cytokine markers of inflammation all returned to preGXT levels by 24 h post GXT.

Conclusion: High intensity exercise results in a transient increase in the expression of inflammatory cytokines in muscle and blood.

Key Words: horse • cytokine • exercise • muscle



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EXERCISE TRAINING-INDUCED HYPERVOLEMIA IN THE HORSE

K.H. McKEEVER, W.A. SCHURG, S.H. JARRETT and V.A. CONVERTINO

The purpose of this study was to determine if a chronic hypervolemia would accompany endurance exercise training in the horse. Six mature previously inactive horses were utilized for this study. During the 5-wk experiment, five of the horses were trained for 14 d on a treadmill ergometer at a constant treadmill speed of 5.6 km hr⁻¹ and a constant grade of 12.5% for graduated lengths of time. One horse was trained by lunging at a trotting pace in a round pen. Following training, plasma volume increased by 4.7 L (29.1%, P<0.05). Although the rate of daily water intake did not change during the training period, 24-h urine output decreased by an average of $3.5 \text{ L} \text{ d}^{-1}$ (-24.5%, P<0.05). Resting glomerular filtration rate and the rate of sodium clearance were not altered by training. However, urea, potassium, and osmotic clearance were decreased by training (P<0.05) while free water clearance was increased (P<0.05). Resting plasma aldosterone and arginine vasopressin concentrations were not altered by training. Plasma potassium concentration was significantly decreased (P<0.05) following the 2 wk training. These data would appear to suggest that renal control mechanisms affecting water substances other than sodium provide the primary route for the training-induced hypervolemia seen in horses.

> *Key Words: exercise* • *plasma volume* • *renal function* • *urea* • *horses*



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FAT-FREE MASS IS RELATED TO ONE-MILE RACE Performance in Elite Standardbred Horses

C.F. KEARNS, K.H. McKEEVER, K. KUMAGAI and T. ABE

This study examined whether body composition was predictive of competitive success in elite Standardbreds (STB). Rump fat and muscle thickness (MTH) (vastus lateralis/intermedius [VL], extensor carpi radialis [ECR]) were measured in vivo in male n=6; female n=8 by B-mode ultrasound. Percentage body fat (%fat) was calculated from rump fat. There were no gender differences for age, body mass (males 432 ± 11 kg; females 443 ± 13 kg), fat-free mass (FFM) (males 400 ± 12 kg; females 400 ± 11 kg), ECR MTH (males 61 ± 2 cm; females 60 ± 2 cm) or race time (RT) (males 113 ± 3 s; females 114 ± 2 s). Males had less (P<0.05) fat mass (males 32 ± 4 kg; females 44 ± 3 kg) and %fat (males $7.4 \pm 0.9\%$; females $9.9 \pm 0.5\%$) and larger (P<0.05) VL MTH (males 88 ± 7 cm; females 81 ± 3 cm). RT was correlated to %fat and fat mass in males (r=0.89; r=0.82, P<0.05) not females (r=0.51; r=0.14). FFM tended to relate to RT in males (r=0.76, P=0.07) and females (r=-0.59, P=0.12). Combined %fat and FFM data were correlated to RT (%fat r=0.70, P<0.01; FFM r=-0.65, P<0.01). RT was not correlated to MTH (VL r=-0.28; ECR r=-0.31). In conclusion, FFM was related to RT in elite STB with %fat negatively related to RT in males.

Key Words: muscle thickness distribution • *ultrasonography* • *horse* • *race performance* • *body composition*



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GLUCOSE INFUSION INCREASES MAXIMAL DURATION OF PROLONGED TREADMILL EXERCISE IN STANDARDBRED HORSES

J.W. FARRIS, K.W. HINCHCLIFF, K.H. McKEEVER and D.R. LAMB

The main purpose of this study was to describe the effect of glucose infusion on selected physiological variables and on the duration of treadmill exercise to fatigue. Six, 3-4 year old, clinically normal Standardbred fillies were used in a randomized and balanced crossover design. The treatments were either an infusion of glucose (an initial bolus followed by an infusion rate adjusted during exercise to maintain glucose at approximately 9.0 mmol/L) or an infusion of a similar volume of normal saline. Saline was also infused during the first 30 min of recovery in both treatments. Treadmill speed and grade were 6.0 m/s and 2°. All horses underwent an exercise and dietary control period for 3 days prior to each treatment. A period of 7-10 days separated the treatments. Treadmill exercise duration was prolonged with glucose (89.8 min \pm 15.4 s.e.m.) compared to saline $(78.7 \pm 12.4 \text{ min})$ (P<0.05). Plasma glucose concentrations were similar at rest and increased (P<0.05) above resting concentrations throughout exercise beginning at 15 min and 35 min for the glucose and saline trials, respectively. By 15 min of exercise, glucose concentrations were 8.72 ± 0.22 and 5.78 ± 0.28 mmol/L (P<0.05) for the glucose and the saline trials, respectively. Changes in haematocrit, plasma lactate, plasma total solids, plasma electrolytes, heart rate, core temperature, body weight and body water loss were similar for both treatments. An explanation for the glucose-induced prolongation of exercise could be the availability of additional substrate during exercise.

> *Key Words: horse* • *glucose* • *endurance* • *exercise*



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HEART RATE RESPONSES OF TWO BREEDS OF FOUR-GAITED HORSES TO A STANDARDISED FIELD GAITED TEST

H.C. MANSO FILHO, H.E.C.C.C. MANSO, K.H. McKEEVER, S.R.R. DUARTE and J.M.G. ABREU

In order to understand how gaited horses use their energy during exercise, a standardised field gaited test (SFGT) was developed to assess energy expenditure of four beat gaited horses independently of size, sex, or breed. This work aimed at developing such an SFGT, using as main measurement parameter the heart rate (HR) of horses during the SFGT performance. Thirty-one four beat gaited horses were evaluated and divided into two groups: FIT (conditioned) and UNFIT (not conditioned). Horses were submitted to the SFGT and their heart rates were measured with a heart rate monitor as follows: right after being mounted, at the beginning of pre-test (HR_{SADDLE}); at 5, 10, 15, 20, 25 and 30 minutes of four beat gait dislocation; and at 15 minutes after the recovery period (T_{+15}) . Maximum HR (HR_{MAX}); HR percentage over 150 beats per minute (HR $_{>150}$), HR percentage over 170 beats per minute (HR_{%>170}), and average HR during the four beat gait stage (HR_{M@M}) of SFGT were calculated. Results were analyzed by ANOVA for repeated measures. Where significant differences were observed, 'T' test was performed and significance was set at 5%. The FIT group presented HR_{MAX}, HR₊₁₅, HR_{M@M}, HR_{%>150} and HR_{%>170} rates lower (P<0.05) than the UNFIT group. It was noted that there was a negative correlation between fitness and HR_{MAX} (R=-0.67; P<0.001) and a positive correlation between HR_{MAX} and HR_{+15} (R=0.60; P<0.001) when comparing the FIT to the UNFIT horses. In conclusion, during the SFGT, the FIT group was more efficient in energy expenditure than the UNFIT group, based on the results observed for the significantly lower HRs during the SFGT. It is relevant to note that the SFGT developed and used in this research, which was easily reproduced and accurate, was able to detect and confirm important adaptations related to fitness in the athletic horse.

> *Key Words: gaited horses* • *energy expenditure* • *standard exercise tests*



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INFLAMMATORY RESPONSES TO THREE MODES OF INTENSE EXERCISE IN STANDARDBRED MARES – A PILOT STUDY

E.D. LAMPRECHT, C.A. BAGNELL and C.A. WILLIAMS

The objective of this study was to compare exercise-induced inflammatory responses in horses undergoing three different treadmill exercise tests. Mares completed a graded exercise test (GXT), an interval exercise test (IET) and a repeated sprint exercise test (RSET). Blood and synovial fluid samples were taken 24 h before (PRE) exercise, 20-30 min, 2 and 24 h following exercise (REC). Blood was analysed for total protein, haematocrit, and total nitrite concentration. Pro-inflammatory and anti-inflammatory cytokine transcripts were measured in whole blood using quantitative real-time polymerase chain reaction. Synovial fluid was analyzed for total nitrite. Mares spent more time at greater than 90% of their maximum heart rate during the RSET than they did for either the GXT or IET. There was an effect of exercise test (P<0.0001) and exercise test by sample interaction (P=0.010) for plasma total nitrite. Pro-inflammatory cytokine transcripts and plasma total protein were elevated (P<0.05) after exercise and there was a higher (P<0.0001) nitrite concentration in tibiotarsal joints compared with radiocarpal joints. As hypothesized, the higher-intensity exercise test (RSET) resulted in greater nitric oxide responses as well as markers of exercise intensity compared with less intense exercise tests (IET, GXT).

Key Words: cytokines • *equine* • *exercise* • *inflammation* • *nitric oxide synovial fluid*



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METABOLIC CHANGES IN FOUR BEAT GAITED HORSES AFTER FIELD MARCHA SIMULATION

E.K. WANDERLEY, H.C. MANSO FILHO, H.E.C.C.C. MANSO, T.A. SANTIAGO and K. H. McKEEVER

Reasons for performing study: Mangalarga-Marchador is a popular 4-gaited Brazilian horse breed; however, there is little information about their metabolic and physiological response to exercise.

Objectives: To measure physiological and metabolic responses of the Mangalarga-Marchador to a simulated marcha field test and to compare these responses between 2 types of marcha gaits (picada and batida).

Methods: Thirteen horses were used in the study and randomly assigned to either the picada or batida gait for the simulated marcha field test (speed ~3.2 m/s; 30 min; load ~80 kg).

Measurements: Included body composition, heart rate (HR), respiratory rate (RR), glucose (GLUC), lactate (LACT), packed cell volume (PCV), total plasma protein (TPP), albumin, urea, creatinine, total and HDL cholesterol, triglycerides, creatine kinase, alanine, glutamate and glutamine (GLN). Measurements were obtained pretest (control/fasting), immediately after simulation (T0), and 15 (T15), 30 (T30), and 240 (T240) min after the simulation. Lactate (LACT) was measured at T0, T15, and T30. Data were analyzed using ANOVA, Tukey's test and *t* tests with significance set at P<0.05.

Results: Significant acute changes were observed in HR, RR, [GLUC], [LACT], [TPP], PCV and [GLN] (P<0.05) relative to control. Heart rate fell below 60 beats/min at T15 and RR recovered to pretest values by T240. Significant increases in [GLUC], [LACT], PCV and [TPP] and a decrease in [GLN] were observed at T0. Treatment and interaction effects were also observed between marcha types and time of sampling for HR, RF, PCV, and [LACT] (P<0.05). These parameters were large in picada.

Conclusion: The simulation of field-test produced changes in some physiological and blood parameters in marcha horses, with some degree of dehydration during recovery period. Also, it was demonstrated that picada horses spend more energy when compared with batida horses at the same speed.

Potential relevance: Batida horses expend less energy when compared with picada horses, which will need special attention in their training and nutritional management.

Key Words: horse • *exercise* • *Mangalarga-Marchador* • *glucose* • *lactate* • *glutamine*



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THE METABOLIC RESPONSE TO TREADMILL GRADED EXERCISE: TRADITIONAL VS. UNDERWATER

P. WATSON, C. MENDONCA, R.A. LEHNHARD, S. TU, S.A. BUTTERFIELD, T. BOUCHARD and K.H. McKEEVER

The purpose of this study was to determine the metabolic effect(s) of four graded exercise tests (GXT) performed on the underwater treadmill (UWT), and compare them to the results from performing the standard Bruce protocol on a traditional land treadmill (LT). Twelve male Division I college athletes performed the stand Bruce protocol on a LT and 4 different GXT protocols on the UWT. Each test was performed to volitional max. oxygen consumption (VO_2) , heart rate (HR), respiratory exchange ratio (RER) and blood lactate (BL), which were measured at regular intervals throughout each of the GXTs. A two-way ANOVA with repeated measures was used to determine any main effect among the variables within the protocols and within the stages. Established effects were identified further using Tukey's post-hoc analysis. VO_2 and HR were positively correlated in all the GXTs, (r=0.992-0.999). When comparing the UWT GXTs to the Bruce, significant $(P \le 0.001)$ differences were found within certain stages for each of the dependent variables. While the Bruce resulted in attainment of VO_{2max} , (mean=55.72 ± 5.92) ml/kg/min), only one of the UWT GXTs resulted in the same level of metabolic stress. At maximum speed and water velocity, the UWT produced a mean VO₂ of 53.9 ± 3.68 ml/kg/min. HR and RER both increased by stage in all the GXTs as did BL. The onset of blood lactate accumulation (OBLA) was identified in all of the GXTs with the exception of one of the UWTs. Mean BL at OBLA ranged from, 3.12 + 1.31 to 5.98 + 4.24 mmol/dl. Our results suggest that an UWT GXT protocol can be developed that would produce metabolic results similar to the Bruce through stage 5. Buoyancy and variation in biomechanics need to be studied further for their effects on energy metabolism while performing work on the UWT. However, this study provides a better understanding of the metabolic demands at different treadmill speeds and water jet settings with the UWT.

Key Words: graded exercise test • underwater running • oxygen consumption • blood lactate • OBLA



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MUSCLE, TENDON, AND SOMATOTROPIN RESPONSES TO THE RESTRICTION OF MUSCLE BLOOD FLOW INDUCED BY KAATSU-WALK TRAINING

T. ABE, C.F. KEARNS, H.C. MANSO FILHO, Y. SATO and K.H. McKEEVER

Objective: The efficacy of KAATSU training has been demonstrated in human athletes, both as a therapeutic method as well as a training aid. The purpose of this study was to investigate the effects of slow walk training combined with restriction of muscle blood flow (KAATSU) on muscle and tendon size.

Methods: Six healthy, unfit Standardbred mares performed walking (240 m/min for 10 min and then 5 min recovery) with KAATSU, and 6 mares performed walking without KAATSA. A specially designed elastic cuff was placed at the most proximal position of the forelegs ad inflated to a pressure of 200-230 mmHg throughout the walking and recovery sessions. The training was conducted once a day 6 days/week for 2 weeks. Skeletal muscle thickness and tendon thickness were measured using B-mode ultrasound at baseline and after 2 weeks of training. Venous blood samples were obtained before the first acute exercise and 5, 15, and 60 min afterwards. Serum somatotropin concentration was determined using a commercially available equine-specific ELISA kit.

Results: The acute increase in plasma somatotropin was 40% greater (P<0.05) in the KAATSU-walk group than in the Control-walk group 5 min after exercise and remained elevated (P<0.05) at 15 and 60 post exercise compared with the Control-walk group. After 2 weeks of training, muscle thickness increased (P<0.05) 3.5% in the KAATSU-walk group but did not change in the Control-walk group (0.7%). Tendon thickness did not change (P>0.05) in either group.

Conclusions: These data demonstrate that KAATSU training can induce muscle hypertrophy in horses and suggest that KAATSU training may provide significant therapeutic/rehabilitative value in horses, as has been shown in man.

Key Words: horse • *blood flow* • *muscle hypertrophy*



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PLASMA β-ENDORPHIN, CORTISOL, AND IMMUNE RESPONSES TO ACUTE EXERCISE ARE ALTERED BY AGE AND EXERCISE TRAINING IN HORSES

K. MALINOWSKI, E.J. SHOCK, P. ROCHELLE, C.F. KEARNS, P.D. GUIRNALDA and K.H. McKEEVER

Reasons for performing study: Ageing appears to affect immune and neuroendocrine function in horses and response to acute exercise. No studies have examined the combined effects of training and ageing on immune and neuroendocrine function in horses.

Hypothesis: To ascertain whether training and age would affect the plasma β -endorphin (BE) and cortisol (C) as well as immune function responses to acute exercise in Standardbred mares.

Methods: Graded exercise tests (GXT) and simulated race tests (SRT) were performed before and after 12 weeks of training at 60% HR_{max} . BE and C were measured at rest and at 5, 10, 20, 40, 60, and 120 min post GXT. Leucocyte cell number, CD4⁺ and CD8⁺ lymphocyte subsets, and mitogen stimulated lymphoproliferative response (LPR), were measured in jugular blood before and after the SRTs.

Results: Cortisol rose by 5 min post GXT in young (Y) and middle-age (MA) mares (P<0.05) and remained elevated until 40 and 60 post GXT, respectively during both pre- and post-training GXT. There was no rise in C in old (O) mares 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). After SRT, 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). The O had reduced LPR to Con A and PHA stimulation (P<0.05) compared to Y and MA after the SRT after both pre- and post-training SRT. LPR to PWM was lower (P<0.05) in O vs. Y and MA after the pre-training SRT. Training caused an increase in resting LPR to PWM in MA only (P<0.05).

Conclusion: Both age and training altered the plasma β -endorphin and cortisol responses as well as and immune responses to acute exercise.

Potential Relevance: This study provides important information on the effects of ageing and training that will aid in the management and care of an increasing number of active older horses.

Key Words: horse • *ageing* • *exercise* • β*-endorphin* • *immune function*



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PLASMA CONSTITUENTS DURING INCREMENTAL TREADMILL EXERCISE IN INTACT AND SPLENECTOMISED HORSES

K.H. McKEEVER, K.W. HINCHCLIFF, S.M. REED and J.T. ROBERTSON

Six intact and 6 splenectomised mares were given an incremental exercise test on a treadmill to examine the fluid and electrolyte changes associated with exercise and the role of the spleen in these changes. Blood samples were obtained at rest and at the end of each 1-min step of the test. Exercise at 7 m/s caused significant (P<0.05) increases in plasma osmolality (intact, +9.9%; splenectomised, +6.2%), plasma protein concentration (intact, +15.8%; splenectomised, +11.4%), and plasma K⁺ concentration (intact, +46%; splenectomised, +57%). Plasma Na⁺ concentration did not increase significantly (P>0.05) in either group (intact, +2.7%; splenectomised, +3.5%).

This appears to be the first record of substantial changes of these constituents during short-term exercise, even before the onset of visible sweat losses. The changes in the concentration of plasma protein suggest that short-term exercise causes a decrease in plasma volume. The changes in the concentration and content of sodium suggest that this is an isotonic shift of fluid. The increase in plasma potassium concentration appears to be due to haemoconcentration as well as an increase in the content of potassium within the vascular compartment.

Key Words: horse • exercise • plasma • protein • osmolality



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PLASMA RENIN ACTIVITY AND ALDOSTERONE AND VASOPRESSIN CONCENTRATIONS DURING INCREMENTAL TREADMILL EXERCISE IN HORSES

K.H. McKEEVER, K.W. HINCHCLIFF, L. SCHMALL, D. LAMB and W. MUIR

Six untrained mares were subjected to incremental treadmill exercise to examine exerciseinduced changes in plasma renin activity (PRA) and plasma aldosterone (ALDO) and plasma arginine vasopressin (AVP) concentrations. Plasma renin activity, ALDO, and AVP concentrations, and heart rate (HR) were measured at each step of an incremental maximal exercise test. Mares ran up a 6° slope on a treadmill set at an initial speed of 4 m/s. Speed was increased 1 m/s each minute until HR reached a plateau. Plasma obtained was stored at -80°C and later was thawed, extracted, and assayed for PRA and ALDO and AVP values by use of radioimmunoassay. Exercise caused significant increase in HR from 40 ± 2 beats/min (mean \pm SEM) at rest to 206 ± 4 beats/min (HR_{max}) at speed of 9 m/s. Plasma renin activity increased from 1.9 ± 1.0 ng/ml/h at rest to a peak of 5.2 ± 1.0 ng/ml/h at 9 m/s, paralleling changes in HR. Up to treadmill speed of 3 m/s, strong linear correlations were obtained between exercise intensity (and duration) and HR (r=0.87, P<0.05) and PRA (r=0.93, P<0.05). Heart rate and PRA reached a plateau and did not increase when speed was increased from 9 to 10 m/s. Plasma ALDO concentration increased from 48 ± 16 pg/ml at rest to 191 ± 72 pg/ml at speed of 10 m/s. Linear relation was found between exercise intensity (and duration) and ALDO concentration (r =0.97, P<0.05). Plasma AVP concentration increased from 4.0 ± 3.0 pg/ml at rest to 95 ± 5.0 pg/ml at speed of 10 m/s. The relation between AVP concentration and exercise intensity (and duration) appeared to be curvilinear, and was described by an exponential function (r=0.92, P<0.05). These data indicate that PRA and ALDO and AVP concentrations increase in horses during progressive treadmill exercise.



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RENAL AND SYSTEMIC HEMODYNAMIC RESPONSES TO SUSTAINED SUBMAXIMAL EXERTION IN HORSES

K.W. HINCHCLIFF, K.H. McKEEVER, L.M. SCHMALL, C.W. KOHN and W.W. MUIR III

We investigated the effects of 1 h of sustained submaximal exertion on the renal and systemic hemodynamics of six horses. The horses ran on a treadmill at a speed that produced heart rates of 55-60% of each horse's maximum heart rate. Exertion produced heart rates of 121 \pm 6.6 and 126 \pm 6.1 (SE) beats/min after 15 and 60 min, respectively. Cardiac output increased significantly (P<0.05) from 70.1 \pm 3.1 to 246.2 \pm 4.7 ml·min⁻¹·kg body wt⁻¹ after 15 min of exertion and thereafter did not change. There was no significant change from rest in *p*-aminohippuric acid and creatinine clearances, filtration fraction, or renal blood flow during exertion. Plasma total solid concentration and hematocrit increased by 3.8 and 8.6%, respectively, between 20 and 60 min of exertion. Pulmonary artery temperature increased significantly from 37.6°C at rest to 38.6°C at 60 min of exertion. This study demonstrates the ability of the horse to maintain renal hemodynamics similar to resting values and systemic hemodynamics at steady-state values despite hemoconcentration and increased body temperature during sustained submaximal exertion.

Key Words: renal blood flow • *p*-aminohippuric acid clearance • creatinine clearance



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RENAL TUBULAR FUNCTION IN HORSES DURING SUBMAXIMAL EXERCISE

K.H. McKEEVER, K.W. HINCHCLIFF, L.M. SCHMALL and W.W. MUIR III

Exercise-induced changes in renal function were examined during steady-state submaximal treadmill exercise in six unfit mares. Horses were randomly assigned to either an exercise or parallel control (no exercise) trial on day 1 and the alternate trial 1 wk later. The mares ran on a treadmill, set at a 6° incline, for 1 h at 55-60% of maximal heart rate. Exercise significantly (P<0.05) increased plasma osmolality, plasma [K⁺], urine flow (+45%), Na⁺ excretion (+371%), K⁺ excretion (+57%), osmotic clearance (+32%), Na⁺ clearance (+391%), K⁺ clearance (+33%), and fractional Na⁺ excretion (+320%) and significantly decreased plasma [Cl⁻], Cl⁻ excretion (-46%), Cl⁻ clearance (-41%), and fractional Cl⁻ excretion (-47%). Glomerular filtration rate, fractional K⁺ excretion, and free water clearance did not change during exercise. Atrial natriuretic peptide increased during exercise from 11 ± 1 pg/ml at rest to a peak of 40 ± 9 pg/ml (264%, P<0.05) at 40 min. Increases in plasma renin activity (66%, P<0.05) were accompanied by increases in plasma aldosterone concentration (760%, P<0.05). Vasopressin concentration increased (P<0.05) steadily over the 60-min period of exercise. It was concluded that, in horses, submaximal exercise-induced increases in urine flow and sodium excretion are associated with a concurrent increase in the plasma concentration of atrial natriuretic peptide.

> Key Words: exertion • atrial natriuretic peptide • plasma renin activity • plasma aldosterone • vasopressin



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RESISTANCE TRAINING-INDUCED INCREASES IN MUSCLE MASS AND PERFORMANCE IN PONIES

R.W. HECK, K.H. McKEEVER, S. ALWAY, W. AUGE, R. WHITEHEAD, A. BERTONE and J.A. LOMBARDO

The purpose of this study was to determine whether 8 wk of progressive resistance exercise training would produce increases in strength and changes in foreleg muscle characteristics indicative of hypertrophy in ponies. Two mature 3- to 6-year old, male ponies $(188 \pm 16 \text{ kg})$ were taught to carry sheets of lead over their saddle region (wither) while walking on a level treadmill at 1.9 m/s. This initial familiarization period was followed by 8 wk of training (3 d per wk), in which the ponies performed a series of progressive sets of weight carrying to fatigue. Each workout started with a 2 min walk at 1.9 m/s followed by sets of weight carrying. The ponies carried 44.5 kg for the first set with increases of 22.3 kg per set until fatigue. Weights were appled and then removed for 60-90 s between setws using a chain hoist and sling apparatus. Measurement of forelimb girth, body weight, and total weight carried were recorded at each workout session. Ultrasoud measurement of the diameters of the superdigital flexor muscles and muscle biopsies were performed before and after the 8 wk training period. Eight weeks of resistance training resulted in significant increases in peak weight carried (260%, P<0.05) and total weight carried (1525%, P<0.05) during each workout. Forelimb girth increased $12 \pm 1\%$ (P<0.05) with a corresponding $19 \pm 3\%$ (P<0.05) increase in muscle crosssectional diameter. There were no changes (P>0.05) in Type 1 muscle fiber area; however, there was a nonsignificant 26% increase in Type IIA + IIB fiber area. These data suggest that 8 wk of progressive resistance exercise training increase strength and cause changes in muscle size and characteristics consistent with hypertrophy.

> *Key Words: strength* • *muscle hypertrophy* • *weightlifting*



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RESTING CONCENTRATIONS OF THE PLASMA FREE Amino acids in horses following chronic Submaximal exercise training

K.H. McKEEVER, W.A. SCHURG, S.H. JARRETT and V.A. CONVERTINO

Six horses were conditioned on a treadmill at a constant speed of 5.6 km/hr on a 12.5% grade for gradually increasing periods of time over 14 days in order to determine the effect of repeated submaximal exercise on the concentrations of plasma free amino acids, protein metabolism, and plasma volume. Following 14-days of training, plasma volume increased (29%, P<0.05), as did total circulating content of plasma protein, albumin and urea. Urinary urea nitrogen excretion decreased (P<0.05) with exercise training. After the first week of training, the concentration of glycine had decreased (P<0.05) and the concentrations glutamic acid, arginine and alanine were increased (P<0.05) when compared to their corresponding pre-training (control week) levels. Compared to pretraining levels, there were decreases (P<0.05) in aspartic acid, histidine, arginine, valine, phenylalanine, isoleucine, and lysine, following the second week of training. Following a week of recovery, all resting concentrations of plasma free amino acids; when compared to their pre-training control; had decreased, with the exception of three nonessential amino acids (glutamic acid, serine, and glycine). Based upon the results of the present study, it would appear that exercise training produced a significant change in the amino acid and protein metabolism of the horse.



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ROLE OF DECREASED PLASMA VOLUME IN HEMATOCRIT ALTERATIONS DURING INCREMENTAL TREADMILL EXERCISE IN HORSES

K.H. McKEEVER, K.W. HINCHCLIFF, S.M. REED and J.T. ROBERTSON

Six intact (IN) and six splenectomized (SP) mares were subjected to an incremental exercise test to examine the role of decreases in plasma volume (PV) in the changes in hematocrit (Hct) normally seen with exercise. Each horse underwent an incremental exercise test in which it ran on a treadmill up a fixed incline of 6°. The test started at a speed of 4 m/s, and speed was increased 1 m/s each min until heart rate reached a plateau. Blood samples were obtained at rest and at the end of the 4, 5, 6, and 7 m/s steps of the exercise test. Resting PV was greater (P<0.05) in the IN group ($28.6 \pm 0.5 \text{ L}$) compared with SP horses (22.7 \pm 2.2 L). Exercise produced a significant increase in Hct in both groups of horses; however, the increases were greater (P < 0.05) at all exercise intensities in IN compared with SP horses. Hct increased rapidly during the first step (4 m/s) in the exercise test in the IN animals. After this, Hct increased slowly in IN horses, and values for the 5, 6, and 7 m/s steps paralleled the changes observed in the SP horses. Corrected Hct values were then used to calculate the relative change in PV. Exercise produced a significant decrease in PV in both groups of horses; however, there were no differences (P>0.05) between the groups when IN horses (-13 ± 3 and $-18 \pm 2\%$) were compared with SP horses (-14 \pm 4 and -17 \pm 4%) at the 6 and 7 m/s steps of the exercise test. These data suggest that the initial increase in Hct in the horse is primarily due to contraction of the spleen and mobilization of red blood cells at the onset of exercise. However, percentage changes in plasma volume, calculated using Hct in the splenectomized horses and the change in PV that was calculated using plasma total solids concentration calculated for both groups of horses, suggest that part of this initial increase in Hct in the intact horses is also due to a 5-10% decrease in PV. Changes in Hct after accommodation of the red cell reserve appear to be due to decreases in PV.

> *Key Words: spleen* • *fluid shifts* • *red cell volume*



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Speed associated with plasma pH, oxygen content, total protein, and urea in an 80-km race

R.M. HOFFMAN, T.M. HESS, C.A. WILLIAMS, D.S. KRONFELD, K.M. GRIEWE-CRANDELL, J.E. WALDRON, P.M. GRAHAM-THIERS, L.S. GAY, R.K. SPLAN, K.E. SAKER and P.A. HARRIS

To test the hypothesis that endurance performance may be related quantitatively to changes in blood, we measured elected blood variables then determined their reference ranges and associations with speed during an 80 km race. The plan had 64 horses in a 2 x 2 factorial design testing a potassium-free electrolyte mix and a vitamin supplement. Blood samples were collected before the race, at 21, 37, 56, and 80 km, and 20 min after finishing, for assay of haematocrit, plasma pH, pO₂, pCO₂, [Na⁺], [K⁺], [Ca⁺⁺], [Mg⁺⁺], [Cl⁻], lactate, glucose, urea, cortisol, α -tocopherol, ascorbate, creatine kinase, aspartate amino transferase, lipid hydroperoxides, total protein, albumin and creatinine, and erythrocyte glutathione and glutathione peroxidase. Data from 34 finishers were analysed statistically. Reference ranges for resting and running horses were wide and overlapping and, therefore, limiting with respect to evaluation of individual horses. Speed correlations were most repeatable, with variables reflecting blood oxygen transport (enabling exercise), acidity and electrolytes (limiting exercise) and total protein (enabling then, perhaps, limiting). Stepwise regressions also included plasma urea concentration (limiting). The association of speed with less plasma acidity and urea suggests the potential for fat adaptation and protein restriction in endurance horses, as found previously in Arabians performing repeated sprints. Conditioning horses fed fat-fortified and protein-restricted diets may not only improve performance by also avoid grain-associated disorders.

> *Key Words: horse* • *endurance exercise* • *speed*



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SPLENECTOMY ALTERS BLOOD PRESSURE RESPONSE TO INCREMENTAL TREADMILL EXERCISE IN HORSES

K.H. McKEEVER, K.W. HINCHCLIFF, S.M. REED and R.L. HAMLIN

Six intact (IN) and five splenectomized (SP) mares were subjected to an incremental exercise test to examine the effects of splenectomy on blood pressure responses during exercise. During the test the horses ran up a 6° grade on a treadmill set at an initial speed of 4 m/s. Speed was increased 1 m/s every 1 min until heart rate (HR) reached a plateau. Hemodynamic data were collected continuously and analyzed for the last 18 s of the 4, 5, 6, and 7 m/s steps of the exercise test. There were no differences (P>0.05) between the groups for resting means for any parameter measured in the study. HR increased (P<0.05) in both groups of horses during exercise with significantly greater HR in the SP horses during the first two steps of the test. There were no differences (P>0.05) in maximal HR between the groups. Right atrial pressure (RAP) did not change during exercise in the SP horses; however, it did increase 20 mmHg (493%, P<0.05) in the IN horses. Right ventricular pressure (RVP) increased (P<0.05) during exercise with no differences (P>0.05) between the groups. Right ventricular maximum change in pressure over time increased (P<0.05) in a similar fashion in both groups of animals. Mean arterial pressure (MAP) increased 29 mmHg (30%, P<0.05) in SP horses, reaching a plateau by the 5 m/s step of the test. In IN horses, MAP continued to increase with treadmill speed and was 71% (-70 mmHg) greater than resting levels at 7 m/s. MAP at 7 m/s was greater (P<0.05) in the IN horses compared with the SP horses. Carotid artery systolic, diastolic, and pulse pressures increased (P<0.05) with exercise intensity in IN horses, whereas only diastolic pressure increased in SP horses. It appears that splenectomy compromises cardiovascular performance during exercise by decreasing the exercise-induced increase in preload.

> *Key Words: hemodynamics* • *baroreceptors* • *cardiovascular function*



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TRAINING-INDUCED ALTERATIONS IN RUMP FAT THICKNESS AND PLASMA LEPTIN CONCENTRATION IN YOUNG MATURE AND OLD STANDARDBRED MARES

J.L. AULISIO, N.R. LIBURT, K. MALINOWSKI and K.H. McKEEVER

The objective of this experiment was to test the hypothesis that ageing and training alter plasma concentrations of the peptide hormone leptin. The rationale for the study is based on prior investigations performed at Rutgers University, where published reports documented that ageing disrupts the immune and endocrine responses to acute exercise. The training period for mature young $(7.3 \pm 0.6 \text{ years}; n=6)$ and old $(22 \pm 0.7 \text{ years}; n=6)$ (mean \pm SE) Standardbred mares was the duration of the summer of 2009 at the Rutgers University Equine Exercise Physiology Laboratory. Mares exercise trained in groups of six in a free-stall motorized circular equine exercise machine for 30 min, three times per week. Each mare performed a graded exercise test before beginning the training, after 8 weeks and after 16 weeks of training. There was no difference in body mass due to age (P>0.10). Training caused an increased body mass in both old and young mares (P<0.10). There was no effect of training on rump fat thickness; therefore, the increase in body mass was primarily due to an increase in fat-free mass (muscle mass). Old mares had significantly lower plasma leptin at all sample points. As a result of training, plasma leptin was reduced in both old and young mares (P<0.10). In conclusion, age and training appear to alter the relationship between body composition and leptin.

> *Key Words: horse* • *ageing* • *training* • *leptin* • *body composition*



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TRAINING-INDUCED ENERGY BALANCE MISMATCH IN Standardbred mares

M.E. GORDON, K.H. McKEEVER, S. BOKMAN, C.L. BETROS, H.C. MANSO-FILHO, N.R. LIBURT and J.M. STRELTSOVA

This study tested the hypothesis that exercise training would alter feed intake (FI), body composition (BC) and plasma concentrations of active ghrelin, leptin, cortisol, insulin and glucose. Eight Standardbred mares (12 ± 2 years, 509 ± 36 kg body weight (BW), mean \pm SD) were trained (EX) in an equine Equi-ciser (initially 3 days per week at 60% maximal heart rate (HR_{max}) for 20 min and gradually increased to 5 days per week at 70% HR_{max} for 30 min, with a 10-min warm-up and 10-min cool-down period at the walk). Six mares (12 ± 2 years, $537 \pm$ 45 kg) served as non-exercise controls (CON). All mares were unfit and had not been subjected to conditioning for 3 years before the experiment. Pre- and post-training incremental exercise tests (GXT) were run to determine HR_{max} and maximal oxygen uptake (VO_{2max}) . A total mixed ration (TMR) of hay cubes was fed free choice for 16 h day⁻¹ with the primary experiment following a 6-week diet adaptation period. Mares' FI was measured daily and reported in grams per kilogram BW of feed eaten per week. Changes in BC were assessed using BW (electronic scale) and percentage fat calculated using rump fat thickness and the Westervelt equation. Blood samples were taken every 2 weeks at 15:25, before mares were given their allotment of hay cubes on a day when they did not exercise, to measure plasma hormone and glucose concentrations. Gastroscopy for gastric ulcers was performed before, during and after the trial. VO_{2max} increased by 7.0% (P<0.03) in EX, but did not change (P>0.05) in CON. FI decreased (P<0.001) in both groups, but was only different (P<0.02) between EX and CON at week 3. Digestible energy (DE) intake (Mcal day⁻¹) was initially higher (P<0.001) than calculated DE requirements in EX. However, over time, DE only matched and then fell below (P<0.03) the DE intake required for training. In CON horses, DE intake was higher (P<0.001) than calculated requirements. BW and percentage body fat increased (P<0.001) over time in EX and CON. Plasma leptin concentration increased (P<0.001) over time in both groups, but was 60% higher (P<0.04) in CON compared to EX at weeks 4–8. There were no differences (P>0.05) in active ghrelin, glucose, insulin or cortisol between the groups and over time. Five out of seven EX mares developed gastric ulcers. No CON mares developed gastric ulcers. Training was associated with changes in plasma leptin concentration, an increased incidence of gastric ulcers and a disruption of the balance between required DE and actual intake.

> *Key Words: energy balance* • *exercise* • *leptin* • *ghrelin* • *equine*



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VARIATIONS IN LACTATE DURING A GRADED EXERCISE TEST DUE TO SAMPLING LOCATION AND METHOD

R.A. LEHNHARD, M. BARTLETT, B.M. ROCHE, K.W. HINCHCLIFF and K.H. McKEEVER

The present study tested the hypothesis that lactate concentration ([La2]) would differ between sample sites and between assay techniques that used different analytical substrates. Six clinically normal adult (two Thoroughbreds, three Standardbreds and one Quarter Horse) mares weighing between 435 and 560 kg were used in the study. Each mare performed an incremental exercise test (graded exercise test, GXT) where it ran on a treadmill at a fixed 6% grade. The GXT started at 3 m/s for 1 min with increased in speed by 1 m/s every 60 s until the horses completed the final 10 m/s step. Jugular vein, pulmonary arterial and carotid arterial blood samples (14 ml) were collected before exercise and during the last 10 s of each step of the GXT. [La2] was measured in whole blood (WB, no manipulations), total blood (TB, where the red blood cells were lysed) and plasma. Data were used to calculate the velocity to produce [La2] of 4 mmol/l (VLA4) and 10 mmol/l (VLA10). Statistical analysis utilized a three-way ANOVA and, where appropriate, the Holm–Sidak or the Student Neuman–Keuls method for post hoc comparisons. The null hypothesis was rejected when P<0.05. There was an effect of exercise intensity on [La2] for all three methods (P<0.001) with all means during exercise significantly greater than the resting mean, and there were differences due to method (i.e. analytical substrate) (P<0.001) and sample site (P=0.043). Comparisons of least-squared means (LSM \pm SE) within site revealed that there was a difference (P<0.05) between jugular vein (5.41 \pm 0.24) and carotid artery (6.24 \pm 0.24) and between carotid and pulmonary artery (5.98 ± 0.24) . There was no difference (P>0.05) between jugular vein and pulmonary artery. Within method, there was a difference (P<0.05) between WB (6.54 \pm 0.36) and TB (5.06 \pm 0.36) and between TB and plasma (6.04 \pm 0.64), but there was no difference (P>0.05) between WB (6.54 ± 0.36) and plasma (6.04 ± 0.64). Further analysis of the data demonstrated that the method and sample site influenced (P<0.05) VLA4 and VLA10.

> *Key Words: equine* • *exertion* • *lactate*



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DEVELOPMENTAL CHANGES IN THE CONCENTRATIONS OF GLUTAMINE AND OTHER AMINO ACIDS IN PLASMA AND SKELETAL MUSCLE OF THE STANDARDBRED FOAL

H.C. MANSO FILHO, K.H. McKEEVER, M.E. GORDON, H.E. MANSO, W.S. LAGAKOS, G. WU and M. WATFORD

Glutamine is concentrated within skeletal muscle, where it has been proposed to play a regulatory role in maintaining protein homeostasis. The work presented here addressed the hypothesis that glutamine would be the most abundant free α -AA in plasma and skeletal muscle in the foal during the first year of life. Glycine, however, was the most abundant free α-AA in plasma at birth and between 3 and 12 mo of age. The concentration of glutamine, the second most abundant AA at birth, increased through the first 7 d (P < 0.05) and then returned to values similar to those at birth. This resulted in glutamine being the most abundant free α -AA in plasma from 1 d through 1 mo of age. The most abundant free α -AA in skeletal muscle at birth was glutamine, but the concentration fell by more than 50% by d 15 and continued to decrease, reaching about one-third of the original values by 1 yr of age (P<0.05). Glutamine synthetase was barely detectable in skeletal muscle at birth, but the abundance increased rapidly within 15 d of birth. The concentration of glycine, the second most abundant α AA in muscle at birth, decreased by about 40% by d 15 (P < 0.05) and then stabilized at this value throughout the year. In contrast, glutamate, alanine, and serine concentrations, the third, fourth, and fifth most abundant free a-AA in muscle at birth, respectively, increased to new stable concentrations between 3 and 6 mo of age (P < 0.05). This resulted in alanine being the most abundant free a-AA in skeletal muscle at 12 mo of age, followed by glutamate, glutamine, and glycine. The decrease in intramuscular glutamine content, particularly during the first 2 wk after birth, is not compatible with a regulatory role for glutamine in muscle protein synthesis because it occurred at the time of maximum growth in these animals. The findings that, at certain times of development, glutamine was not the most abundant free α -AA in the foal is novel and signifies that intramuscular glutamine may have functions specific to muscle type and mammalian species.

Key Words: amino acid • body composition • glutamine, • glutamine synthetase • horse • skeletal muscle



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NITROGENOUS CONSTITUENTS IN PLASMA OF FOALS WEANED AT THREE, FOUR, OR SIX MONTHS OF AGE

S.H. JARRETT, K.H. McKEEVER, B.L. REID and W.A. SCHURG

A study was conducted to measure the nitrogenous constituents in the blood of foals weaned at 3, 4, or 6 months of age (four foals per weaning age). Jugular blood was sampled from one week prior to weaning until one month post-weaning, and was analyzed for total protein, albumin, creatinine, urea Nitrogen and free amino acid content. Results indicated that the concentrations of most of the constituents in the blood of foals were not altered by the age of weaning, however, free amino acids decreased as age at weaning increased and creatinine was significantly higher in foals weaned at 6 months of age. Plasma creatinine, lysine, and methionine levels generally decreased between pre-weaning and one month post-weaning. These data establish baseline values for blood nitrogenous constituents in foals at weaning.



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TEMPORAL FOLATE STATUS DURING LACTATION IN MARES AND GROWTH IN FOALS

A.L. ORDAKOWSKI-BURK, D.S. KRONFELD, C.A. WILLIAMS, L.S. GAY and D.J. SKLAN

Objective: To identify changes in folate status of mares and foals during lactation and growth, respectively.

Animal: 20 Thoroughbred mares and foals.

Procedures: Pregnant mares, and following foaling the same mares with their foals, were maintained on mixed grass-legume pasture and fed either a traditional dietary supplement rich in sugar and starch (SS) or a dietary supplement high in fat and fiber (FF). Blood samples were collected monthly from mares and foals up to 6 months after foaling. Total folate concentration in feed and forage was determined. Analyses of plasma folate, RBC folate, plasma homocysteine (HCY), and milk folate concentrations were performed.

Results: Mare plasma folate concentrations declined moderately during 6 months of lactation. Mare RBC folate concentrations initially increased after foaling up to 3 months but declined toward the end of the study. Plasma HCY concentration was higher for mares fed the SS supplement, compared with mares fed the FF supplement from foaling to 6 months of lactation. Milk folate concentrations decreased during the first 3 months and then increased. Foal plasma folate initially declined but then increased. Stable concentrations of RBC folate were observed in foals. Plasma HCY concentrations in foals were unaffected by growth during the last 5 months. References range values for plasma folate, RBC folate, milk folate, and plasma HCY concentrations in healthy lactational mares and young growing foals were determined.

Conclusions and Clinical Relevance: Folate status was not impaired in lactating mares and growing foals under the conditions in our study. It appears that folate supplementation is not necessary.



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ACUTE VASCULAR OCCLUSION IN HORSES: EFFECTS ON SKELETAL MUSCLE SIZE AND BLOOD FLOW

T. ABE, C.F. KEARNS, H.C. MANSO FILHO, Y. SATO, M. SLEEPER and K.H. McKEEVER

The purpose of this study was to demonstrate whether acute vascular occlusion was safe and if it would result in changes to limb muscle size in horses. Six healthy, unfit Standardbred mares were used. Horses (standing at rest) wore an occlusion cuff at the most proximal position of the left forelimb. The right forelimb was used as control. An occlusion pressure of 200mmHg was set for 5 min followed by a 2 min recovery. Three sets of occlusions were given to each horse. Muscle thickness was measured using B-mode ultrasound. The circumference of the forelimb and first phalanx was measured using a flexible tape measure. Pulsed-wave Doppler was performed on the radialis artery with a 5-10 MHz mechanical transducer at baseline and at each occlusion. Peak flow velocity (PFV) and the flow velocity integral (FVI) were measured each time. Mid-forelimb, but not first phalanx, girth was increased (P<0.05) in the occluded but not in the control leg following occlusion. Extensor and flexor muscle thickness was increased (P<0.05) in the occluded but not in the control leg. There were no changes (P>0.05) in PFV or FVI at any measurement time point. Acute vascular occlusion may be a suitable and safe model for studying muscle hypertrophy in horses.



ADIPONECTIN AND LEPTIN ARE RELATED TO FAT MASS IN HORSES

C.F. KEARNS, K.H. McKEEVER, V. ROEGNER, S.M. BRADY and K. MALINOWSKI

Plasma concentrations of adiponectin and leptin were measured in 23 mature Standardbred mares (age: 10 ± 3 years) and 12 weanling fillies (10 Quarter Horse/Belgian crossbreds and two Quarter Horses; aged: 4 ± 3 months) to test the hypothesis that adipocytokines are proportional to adiposity in horses. Rump fat thickness was measured using B-mode ultrasound and percent body fat (% fat) calculated using a published formula for the estimation of fatness in horses. Plasma adiponectin and leptin were determined using radioimmunoassay. In the absence of purified equine adiponectin or leptin, results were expressed as human equivalents (HE) of immunoreactive (ir) adipocytokines. Plasma ir-leptin HE concentration was positively correlated (r=0.543; P<0.001) with percent body fat and (r=0.556; P<0.001) to fat mass in all horses. The plasma ir-leptin HE concentration was lower (P=0.03) in weanlings $(1.90 \pm 0.34 \text{ ng/mL})$ HE) than in mature mares $(3.47 \pm 0.50 \text{ ng/mL HE})$. The ratio of ir-adiponectin HE to irleptin HE was negatively correlated (r=-0.621; P<0.001) to percent fat and (r=-631; P<0.001) to fat mass in all horses. The ratio of ir-adiponectin HE to ir-leptin HE was higher (P<0.001) in weanlings (3.04 ± 0.51) than mature mares (1.03 ± 0.13) . These data suggest that leptin is proportional while adiponectin is inversely proportional to adiposity in horses.

> *Key Words: adipocytokines* • *equine* • *body composition*



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CHANGES IN GLUTAMINE METABOLISM INDICATE A MILD CATABOLIC STATE IN THE TRANSITION MARE

H.C. MANSO FILHO, K.H. McKEEVER, M.E. GORDON, H.E.C. COSTA, W.S. LAGAKOS and M. WATFORD

Glutamine is the most abundant free α -AA in the mammalian body, and large amounts of glutamine are extracted by both the fetus during pregnancy and the mammary gland during lactation. The work presented here addressed the hypothesis that there would be major changes in glutamine metabolism in the mare during the transition period, the time between late gestation, parturition, and early lactation. Eight foals were born to Standardbred mares provided with energy and protein at 10% above NRC recommendations, and foals remained with mares for 6 mo. During lactation, lean body mass decreased by 1.5% (P<0.05), whereas fat mass was unchanged throughout gestation and lactation. There was a sharp increase in the concentration of most plasma metabolites and hormones after birth, which was due in part to hemoconcentration because of fluid shifts at parturition. Plasma glutamine concentration, however, was maintained at greater concentrations for up to 2 wk postpartum but then began to decrease, reaching a nadir at approximately 6 wk of lactation. Skeletal muscle glutamine content did not change, but glutamine synthetase expression was decreased at the end of lactation (P < 0.05). Free glutamine was highly abundant in milk early in lactation, but the concentration decreased by more than 50% after 3 mo. of lactation and paralleled the decrease in plasma glutamine concentration. Thus, lactation represents a mild catabolic state for the mare in which decreased glutamine concentrations may compromise the availability of glutamine to other tissues such as the intestines and the immune system.

> Key Words: body composition • equine • glutamine • glutamine synthetase • lactation • pregnancy



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CHRONIC LAMINITIS IS ASSOCIATED WITH POTENTIAL BACTERIAL PATHOGENS IN THE LAMINAE

J.C. ONISHI, J. PARK, M.M. HAGGBLOM, M.J. FENNELL and M.N. FUGARO

A common sequella of chronic laminitis in horses is repeated abscesses with variable lameness and drainage. It is unclear whether the exudate represents the debridement phase of a non-septic inflammatory process involving clearance of laminar tissue damaged during the acute episode of laminitis, or a response to a microbial infection developed by ascent of microbes from the environment to the tissue via the white line. The objective of this study was to evaluate the possibility that an undiagnosed microbial infection in laminar tissue is present in laminar tissue collected from chronically laminitic horses without an active hoof abscess. Methods to collect laminar tissue, aseptically, from control (non-laminitic) horses and those with chronic/recurrent laminitis are described. Laminae homogenates were evaluated for the presence of bacteria. Bacteria were identified using biochemical tests and sequencing of 16S rRNA and virulence genes. Laminae from chronically lamintic horses revealed 100-fold higher levels (P=0.002) of bacteria compared to control, non-laminitic horses. Although environmental organisms were identified, potential pathogens were identified. Included were Gram positive bacteria, Brevibacterium luteolum, coagulase-negative Staphylococcus spp. as well as Gram negative bacteria, enterohemorrhagic Escherichia coli and Alcaligenes faecalis. Further research is warranted to evaluate the role of bacteria in equine chronic laminitis.

> *Key Words: chronic laminitis* • *equine laminar tissue* • *bacterial pathogens*



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EFFECT OF DIFFERENT SEPARATION PROTOCOLS BETWEEN MARES AND FOALS ON PLASMA CORTISOL AND CELL-MEDIATED IMMUNE RESPONSE

K. MALINOWSKI, N.A. HALLQUIST, L. HELYAR, A.R. SHERMAN and C.G. SCANES

The objective was to compare effects of weaning in pairs of singly on physiological indicators of stress (plasma cortisol [F]) and lymphocyte proliferation response to mitogens expressed as stimulation index [S.I.] in mares and foals. Twenty pairs of Standardbred mares and foals were assigned to treatment groups as follows: non-weaned (control), n=6; weaned singly (WS), n=6; weaned pairs (WP), n=8. Blood sampling to determine cortisol was performed via jugular venipuncture prior to weaning and at 4, 8, 16, 24, 32, 40, and 48h post-weaning. Blood samples for lymphocyte blastogenesis in response to Concanavalin A (Con A) or Phytohemagglutinin (PHA) were taken prior to, 4 and 28 h post-weaning.

There was a trend toward increased F concentrations post-weaning in both mares and foals. WS and WP foals displayed increased F concentrations for up to 40 hours post-weaning when compared to control foals (48.8, 59.0, 22.1 ng/ml, respectively). Weaned mares displayed elevated F concentrations compared to controls for up to 24 hours post-weaning (65.9 and 40.5 ng/ml, respectively).

There was less (P<0.05) lymphocyte proliferation in weaned mares compared to controls in response to stimulation by both Con A and PHA. There was also lower (P<0.05) lymphocyte proliferation response to mitogens in WP foals compared to control foals and WS foals. There was no difference (P>0.05) between WS or control foals.

While weaning caused an increase in plasma F concentrations, there was no difference in that increase due to the effect of separation protocol. Lymphocyte proliferation in response to mitogen was lessened in weaned mares and WP foals. The traditional management practice of paired weaning appears to be more stressful than weaning singly, perhaps through aggressive behavior by a dominant partner.



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EQUINE PLACENTA EXPRESSES GLUTAMINE SYNTHETASE

H.C. MANSO FILHO, H.E. COSTA, G. WU, K.H. MCKEEVER and M. WATFORD

In most mammalian species the developing fetus utilizes large amounts of glutamine derived both from the maternal circulation and synthesized de novo in the placenta. The present study was designed to determine the role of the placenta in glutamine synthesis in the horse. The placentae from eight Standardbred mares were sampled immediately after parturition together with additional tissues obtained at necropsy from three Standardbred mares during diestrous. Glutamine synthetase protein was detectable in the non-pregnant horn of the placenta in amounts similar to those seen in gluteus muscle, but the amount in the pregnant horn was two times greater than in the non-pregnant horn. Glutamine was the second most abundant amino acid in amniotic fluid at a concentration of $310 \pm$ 26μ mole/L with that of glycine being $535 \pm 48 \mu$ mole/L. The most abundant amino acids in placental tissue were glycine $(3,732 \pm 194 \mu$ mole/Kg), glutamate $(3,500 \pm 343 \mu$ mole/Kg) and glutamine $(2,836 \pm 208 \mu$ mole/Kg). The results illustrate the importance of glutamine to the equine fetus and establish that the placenta, particularly the pregnant horn, has considerable capacity for glutamine synthesis.

> Key Words: amino acids • equine, horse • glutamine • glutamine synthetase • placenta



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EVALUATION OF WASHING WITH COLD WATER TO FACILITATE HEAT DISSIPATION IN HORSES EXERCISED IN HOT, HUMID CONDITIONS

C.W. KOHN, K.W. HINCHCLIFF and K.H. MCKEEVER

Objective: To determine whether body temperature of horses exercised in hot, humid conditions and then repetitively washed with cold water will decrease more rapidly than that of horses that are not washed, and to determine whether washing with cold water has deleterious effects on horses. ANIMALS: 5 physically fit Thoroughbred mares, 3 to 10 years old.

Procedures: Horses were exercised on a high-speed treadmill in hot $(31.1 \pm 0.3^{\circ}C)$, humid (relative humidity, 77.7 ± 2%) conditions. Exercise was terminated when pulmonary artery temperature reached 41.5°C. Values for pulmonary artery, rectal, and left gluteal muscle temperatures were compared throughout a 30-minute recovery period after exercise during which horses stood quietly (passive cooling) or were cooled (active cooling) by repeated applications of cold (1 5.6 ± 0.6°C) water.

Results: Pulmonary artery temperature was significantly less for actively cooled horses, compared with passively cooled horses 4 minutes into the recovery period. Left gluteal muscle temperature decreased significantly in actively cooled, but not passively cooled, horses during the recovery period. Heart rate and rectal temperature were significantly less for actively cooled horses by 15 minutes of the recovery period. Cooling technique did not affect hydration status, muscle health, or serum electrolyte concentrations. Active cooling did not cause obvious adverse effects.

Conclusion and Clinical Relevance: Active cooling by washing with cold water is a safe, effective means for facilitating heat dissipation of horses after exercise in a hot, humid environment.



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HORSE HAIR COAT CLEANLINESS IS AFFECTED BY BEDDING MATERIAL: A COMPARISON OF CLEAN AND USED WHEAT STRAW, WOOD SHAVINGS AND PELLETED NEWSPAPER

J. MCCLAIN, J.E. WOHLT, K.H. McKEEVER and P.M. WARD

Wheat straw, wood shavings and pelleted recycled newspaper were used as bedding materials and compared in maintaining horse hair coat cleanliness. Each material was sampled prior to use and after use having been wetted with urine in a bedding study conducted with 4 adult horses in box stalls. Because feces were removed daily during stall maintenance, freshly voided feces were also collected so that all components in a used bedding (clean portion, wetted portion, feces) were represented. Hair coat cleanliness was measured in vitro by exposing a tanned pony hide with hair to clean and wet beddings as well as feces. Cleanliness was evaluated in terms of the amounts of moisture, volatile-N and solid matter transferred from each component and type of bedding material to the haired hide. Transfers to wool flannel were also measured as a comparison to the haired hide. Woolen products are used in the horse industry and also come into contact with components of used bedding. Freshly voided feces and used beddings transferred more (P<0.05) moisture (85 and 54%, respectively) than clean beddings. Wheat straw transferred less (P<0.05) solid matter (86 and 64%, respectively) than wood shavings or pelleted newspaper. Wood shavings transferred more (P<0.05) solid matter than wheat straw or pelleted newspaper to wool flannel resulting in a statistically significant used bedding X surface interaction. These data indicate that type as well as components of used beddings affect hair coat cleanliness. The ability of a bedding material to maintain hair coat cleanliness should also be a factor (e.g., cost, availability, moisture holding capacity, nontoxicity) in its consideration for use as a bedding for animals.



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ILLUMINA SEQUENCING OF THE V4 HYPERVARIABLE REGION 16S RRNA GENE REVEALS EXTENSIVE CHANGES IN BACTERIAL COMMUNITIES IN THE CECUM FOLLOWING CARBOHYDRATE ORAL INFUSION AND DEVELOPMENT OF EARLY-STAGE ACUTE LAMINITIS IN THE HORSE

M.M. MOREAU, S.C. EADES, C.R. REINEMEYER, M.N. FUGARO and J.C. ONISHI

In the equine carbohydrate overload model of acute laminitis, disease progression is associated with changes in bacteria found in the cecum. To date, research has focused on changes in specific Gram-positive bacteria in this portion of the intestinal tract. Metagenomic methods are now available making it possible to interrogate microbial communities using animal protocols that sufficiently power a study. In this study, the microbiota in cecal fluid collected from control, non-laminitic horses (n=8) and from horses with early-stage acute laminitis induced with either oligofructan (n=6) or cornstarch (n=6) were profiled. The microbiota were identified based on sequencing the V4 hypervariable region of the 16S rRNA gene. The results of the study show that the relative abundance of Lactobacillus sp. and Streptococcus sp. increased significantly (P<0.05) following OF and CS infusion. Other significant changes included an increase (P<0.05) in relative abundance of Veillonella sp. and Serratia sp., two potentially pathogenic, Gram-negative bacteria. Significant decreases in the relative abundance of presumptive normal flora were detected as well. Although changes in cecal microbiota described in this communication are from a pilot study, it is hypothesized that an overgrowth of pathogenic Gram-negative bacteria develops and contributes to enterocolitis, pyrexia and lameness in the carbohydrate overload model of acute laminitis.



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INFLAMMATORY AND REDOX STATUS OF PONIES WITH A HISTORY OF PASTURE-ASSOCIATED LAMINITIS

K. TREIBER, R. CARTER, L. GAY, C. WILLIAMS and R. GEOR

Inflammatory and redox signals could render lamellar tissue susceptible to damage and contribute to higher risk for laminitis in obese or insulin resistant ponies just as these factors contribute to health risks in humans with metabolic syndrome. This study evaluated circulating markers of inflammatory and redox status in ponies that had a history of recurrent bouts of pasture-associated laminitis (PL, n=42) or had never developed clinical laminitis (NL, n=34) under the current management conditions. There were no differences (P>0.05) between PL and NL ponies for markers of antioxidant function (glutathione, glutathione peroxidase, superoxide dismutase) or increased oxidative pressure (malondialdehyde, apoptosis, 3-nitrotyrosine). Inflammatory status, as indicated by fibrinogen concentration, was also not different between pony groups (P=0.84). However, PL ponies had higher (P<0.001) plasma concentrations of the proinflammatory cytokine TNF- α than NL ponies. This suggests that a predisposition to laminitis is associated with increased circulating inflammatory cytokines. TNF-α could also represent a contributing factor to increased insulin resistance observed in laminitis prone ponies. These results provide new insight into potential mechanisms and risk factors underlying laminitis.

Key Words: laminitis • *inflammation* • *oxidative stress* • *tumor necrosis factor-*α



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INTESTINAL BACTERIAL OVERGROWTH INCLUDES POTENTIAL PATHOGENS IN THE CARBOHYDRATE OVERLOAD MODELS OF EQUINE ACUTE LAMINITIS

J.C. ONISHI, J. PARK, J. PRADO, S.C. EADES, M.H. MIRZA, M.N. FUGARO, M.M. HAGGBLOM and C.R. REINEMEYER

Carbohydrate overload models of equine acute laminitis are used to study the development of lameness. It is hypothesized that a diet-induced shift in cecal bacterial communities contributes to the development of the pro-inflammatory state that progresses to laminar failure. It is proposed that vasoactive amines, protease activators and endotoxin, all bacterial derived bioactive metabolites, play a role in disease development. Questions regarding the oral bioavailability of many of the bacterial derived bioactive metabolites remain. This study evaluates the possibility that a carbohydrateinduced overgrowth of potentially pathogenic cecal bacteria occurs and that bacterial translocation contributes toward the development of the pro-inflammatory state. Two groups of mixed-breed horses were used, those with laminitis induced by cornstarch (n=6) or oligofructan (n=6) and non-laminitic controls (n=8). Cecal fluid and tissue homogenates of extra-intestinal sites including the laminae were used to enumerate Gram-negative and -positive bacteria. Horses that developed Obel grade 2 lameness, revealed a significant overgrowth of potentially pathogenic Gram-positive and Gramnegative intestinal bacteria within the cecal fluid. Although colonization of extraintestinal sites with potentially pathogenic bacteria was not detected, results of this study indicate that cecal/colonic lymphadenopathy and eosinophilia develop in horses progressing to lameness. It is hypothesized that the pro-inflammatory state in carbohydrate overload models of equine acute laminitis is driven by an immune response to the rapid overgrowth of Gram-positive and Gram-negative cecal bacterial communities in the gut. Further equine research is indicated to study the immunological response, involving the lymphatic system that develops in the model.

Key Words:

equine • carbohydrate overload model • acute laminitis • bacterial overgrowth • bacterial translocation • lymphadenopathy



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INTRAPERICARDIAL DENERVATION: RADIAL ARTERY BLOOD FLOW AND HEART RATE RESPONSES TO LBNP

K.H. McKEEVER, M.G. SKIDMORE, L.C. KEIL and H. SANDLER

Eight rhesus monkeys were used to study responses of radial artery blood flow velocity (RABFV) and heart rate (HR) to low (0 to -20 mmHg) and high (0 to -60 mmHg) ramp exposures during supine lower body negative pressure (LBNP). These levels were chosen to separate peripheral vascular responses associated with stimulation of low- and highpressure baroreceptors. Four monkeys had efferent and afferent cardiac denervation by use of the Randall procedure with pharmacological (phenylephrine and atropine) verification. Animals were studied 3 wk after surgery to avoid reinnervation. Findings were compared with those of four identically treated intact animals. Denervated animals showed no change in RABFV or HR during low-level LBNP; however, HR increased significantly (P<0.05) when LBNP reached -50 mmHg and blood flow velocity also fell (P<0.05) starting at -30 mmHg pressure. In contrast, intact animals showed steady decreases in RABFV during both high- and low pressure protocols, with HR showing a 6beat/min increase (P<0.05) starting at -20 mmHg pressure. As with denervated animals, intact animals showed a more pronounced increase in HR after reaching a level of -60mmHg suction. Cardiac output (electromagnetic flowmeter, ascending aorta) fell significantly in both groups starting at -30 mmHg pressure. Left ventricular pressure (Konigsberg pressure cell) in three intact animals showed a progressive fall in systolic pressure starting at -10 mmHg suction, which became significant at -55 mmHg pressure. These results demonstrate that cardiac denervation by use of the Randall technique significantly affects RABFV and HR responses to LBNP in rhesus monkeys. The lack of RABFV change during LBNP in denervated animals suggests that these changes coupled with HR response can be used as an effective method to verify the completeness of denervation of low-pressure baroreceptors in animals that have undergone intrapericardial denervation.

> *Key Words: baroreceptors* • *cardiac denervation*



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MATERNAL AND FOETAL HEART RATES DURING EXERCISE IN HORSES

R.A. LEHNHARD, H.C. MANSO FILHO, R.C. CAUSEY, M.WATFORD and K.H. McKEEVER

This study tested two hypotheses. First, that moderate exercise would alter maternal and foetal heart rate (HR) in the horse. Second, that pregnancy would alter the HR, plasma cortisol and plasma lactate response to moderate exercise in mares. Six unfit, pregnant Standardbred mares (6-19 years) underwent two incremental graded exercise tests (GXT). The first GXT was performed at approximately 9 months of gestation, which represents c. 80% of the total pregnancy time, and the second GXT at approximately 6 months post-parturition. During the GXT, mares ran on a treadmill up a fixed 6% incline completing three 1 min steps at velocities of 4, 6 and 7ms21. Maternal HR was obtained via both electrocardiograph (ECG) and the ventricular waveform obtained from a micromanometer catheter. Data were recorded at rest, at 4, 6 and 7ms21 of the GXT and at 0, 1, 2, 3, 4 and 5 min post-GXT. Foetal HR rate was measured via ECG before, immediately after and at 1, 2, 3, 4 and 5 min post-GXT. Radioimmunoassay (RIA) kits were used to measure plasma cortisol concentration in samples collected before the GXT, immediately after exercise and after 5 min of recovery. Plasma lactate concentrations (LA) were measured at rest, at 4, 6 and 7m/s and at 5 min post-GXT. Plasma cortisol concentrations were measured using RIA and the samples collected at rest, immediately after and at 5 min post-GXT. Exercise caused no change (P>0.05) in foetal HR. Maternal HR, plasma LA and plasma cortisol concentration were greater (P<0.05) during the postparturient GXT compared with the pre-parturition GXT. Body weight (mean \pm SE) was lower after parturition (570 \pm 20 vs. 505 \pm 22 kg; P<0.05). Consequentially, there were differences (P<0.05) in work rate (watts) at each step of the GXT (1557 ± 53 vs. 1381 ± 60 ; 2339 ± 80 vs. 2071 ± 90 ; and 2571 ± 121 vs. 2416 ± 105 W). These data suggest that mares benefit from greater cardiovascular efficiency during pregnancy. Additionally, the lack of a change in foetal HR suggests that the unborn foal is not stressed during moderate maternal exercise.

> *Key Words: horse* • *heart rate* • *exercise* • *pregnancy* • *cortisol* • *lactate*



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NEUROENDOCRINE CONTROL OF BLOOD VOLUME, BLOOD PRESSURE AND CARDIOVASCULAR FUNCTION IN HORSES

K.H. McKEEVER and K.W. HINCHCLIFF

Several body systems make critical contributions to the defense of blood volume, mean arterial pressure and plasma tonicity. These mechanisms ensure adequate blood flow to the working muscles and vital tissues along with the provision of adequate fluid volume for sweating and thermoregulation. The present paper integrates data from several recent studies that examined the neuroendocrine control of cardiovascular function in exercising horses. These studies focused on the effects of exercise on plasma concentrations of atrial natriuretic peptide (ANP), arginine vasopressin (AVP), plasma renin activity (PRA) and aldosterone (ALDO), hormones associated with the regulation of blood pressure, the distribution of blood flow and the long term control of vascular fluid volume. Data were collected during incremental and endurance exercise, following frusemide administration and after splenectomy. Increases in AYP during exercise were associated with increased atrial pressure and stretching of the atrium. Increases in PRA and ALDO are correlated with increases in sympathetic activity during incremental exercise and with decreases in plasma Na⁺ and CI⁻ concentrations. Plasma AVP increases later in exercise in response to fluid losses, increases in plasma osmolality, and decreases in right atrial pressure. AVP has a minimal effect on renal function during exercise and may be more important during recovery, when it stimulates thirst and drinking. Frusemide and splenectomy decrease atrial pressure, resulting in lower plasma ANP and greater plasma AVP concentrations during steady-state submaximal exercise. The ANP and AVP responses are part of the neuroendocrine action of the cardiopulmonary baroreflex which appears to mediate the accommodation of the mobilized splenic reserve in the horse. In summary, exercise induced increases in ANP, AVP, PRA and ALDO in the horse appear to play an important role in cardiovascular homeostasis.

Key Words:

horse • exercise • catecholamines • atrial natriuretic peptide • vasopressin •plasma renin activity • aldosterone



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OVERVIEW OF HORSE BODY COMPOSITION AND MUSCLE ARCHITECTURE: IMPLICATIONS FOR PERFORMANCE

C.F. KEARNS, K.H. McKEEVER and T. ABE

Locomotion requires skeletal muscle to sustain and generate force. A muscle's force potential is proportional to its weight. Since the larger the muscle the larger its potential power output, a better understanding of the proportion of skeletal muscle a horse possesses may lead to a better understanding of horse performance. Several techniques exist to assess body composition, which include dual energy X-ray absorption, underwater (hydrostatic) weighing, derivation from total body water, bio-electric impedance, air displacement, body condition scoring, cadaver dissection and ultrasound. The relevance of each method to the equine industry will be discussed as will the practical information that the existing horse body composition studies have provided. Attention will be given to the data regarding the implications of body composition on the performance horse. The limited number of studies discussing different varieties of muscle architectures and the functional importance of these muscles will also be addressed. These body composition data may provide a better understanding of important issues in horse care that can lead to more optimal horse care techniques and a healthier and safer environment for horses.

> *Key Words: muscle mass* • *fat mass* • *muscle architecture* • *performance*



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PLASMA ALDOSTERONE CONCENTRATION AND RENAL SODIUM EXCRETION ARE ALTERED DURING THE FIRST DAYS OF TRAINING

K.H. McKEEVER, R. SCALI, S. GEISER and C.F. KEARNS

The purpose of the present study was to determine whether the training-induced hypervolaemic response seen in the horse is associated with aldosterone-mediated renal mechanisms affecting sodium conservation during the first days of training. Five healthy, Standardbred mares (weight 450-500 kg, age 4-8 years) that were unfit, but accustomed to running on the treadmill, were used to test the hypothesis that repeated submaximal exercise would alter plasma aldosterone (ALDO) concentration and renal excretion of electrolytes in horses within the first 3 days of training. The experiment consisted of a 2 week housing equilibration period followed by a 1 week control period and a 3 day exercise training period (30 min/day at 60% VO_{2max}). During control, ALDO and renal fluid and electrolyte losses were measured for 24 h on 3 separate days. Renal function (urine volume [UV], 24 h excretion of Na⁺, K⁺, and Cl⁻ [U_{NA+}V, U_{K+}V, U_{Cl}·V], clearance of Na⁺ [C_{NA+}], K⁺ [C_{K+}] and Cl⁻ [C_{Cl-}], creatinine [C_{Cr}], osmotic substances [C_{osm}], and solute-free water [FWC], and the fractional excretion of Na⁺, K⁺, and Cl⁻) and ALDO were measured for an additional 3 consecutive days during the training period. There were no differences (P>0.05) in any variable during the control period. Plasma volume increased (+18.7%, P<0.05) after 3 days of training. During training, there were no significant changes in plasma osmolality, electrolyte concentrations or C_{Cr}. Training caused decreases (P<0.05) in UV (-30%), U_{NA+}V (-73%), $U_{K+}V$, (-55%) and $U_{Cl-}V$ (-70%). Training also caused decreases (P<0.05) in C_{osm} (-30%), through decreases in CNa+ (-60%), CK+ (-60%), and CCl- (-66%). Interestingly, FWC increased (+30%, P<0.05), whereas, there were significant decreases in the fractional excretion of Na⁺ (-59%), K⁺ (-48%) and Cl⁻ (-60%). Training caused substantial elevations in both pre-exercise (967%, P<0.05) and post-exercise (+3013%, P<0.05) plasma ALDO concentrations suggesting an increase in both basal levels and the responsiveness to acute exercise. Together, these observations suggest that mechanisms affecting tubular conservation of electrolytes contribute to the early response to training. However, it is also concluded that renal mechanisms appear to be only part of the mechanism for conserving sodium and water intake as well as training-induced changes in gastrointestinal mechanisms affecting electrolyte and water balance.

> Key Words: horse • hypervolaemia • plasma volume • aldosterone • renal sodium • training





RELATIONSHIP BETWEEN BODY COMPOSITION, BLOOD VOLUME AND MAXIMAL OXYGEN UPTAKE

C.F. KEARNS, K.H. MCKEEVER, H. JOHN-ALDER, T. ABE and W.F. BRECHUE

It has long been known that body mass and, more specifically, lean body mass are strongly correlated with maximal oxygen uptake (VO_{2max}) in man and animals. However, there are no data to date describing this phenomenon in the horse. The purpose of this paper is to examine the relationship between body composition and VO_{2max} in the horse. Twenty-three healthy and unfit Standardbred mares performed an incremental exercise test (GXT) to measure VO_{2max}. Rump fat thickness (RTH), a measure of fat covering, was measured using B-mode ultrasound. Plasma volume, total blood volume and red cell volume were determined, using the Evan's Blue dye dilution technique and packed cell volume. VO_{2max} was correlated with body mass (r=0.541; P<0.01) and exercise haematocrit (exHCT; r=0.407; P<0.05) but not RTH or the other haematological variables. To eliminate the influence of body mass on the individual variables, a regression analysis was performed on the mass-residuals of VO_{2max}, RTH, plasma volume and exHCT. The residuals of VO_{2max} were correlated negatively with the residuals of RTH (r=-0.687; P=0.0003) and positively with the residuals of exHCT (r=0.422; P=0.045) but not plasma volume. VO_{2max} could be predicted from a linear combination of the residuals of RTH and exHCT (r=0.767; P<0.0001). These data indicate that VO_{2max} in the horse is significantly related to fat-free mass (FFM), independent of body mass. Red blood cells from the splenic reserve constitute an important factor in the horse's ability to achieve a high VO_{2max}. Therefore, lean body mass may be a more appropriate basis for assessing metabolic function in the athletic horse.

> Key Words: horse, exercise • aerobic capacity • equine • allometry • fat free mass (FFM)



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VENOGRAMS FOR USE IN LAMINITIS TREATMENT

S. EASTMAN, F. REDDEN and C.A. WILLIAMS

Venography is a very valuable tool that can be used in the diagnosis and treatment of laminitis. It can be used to assess vascular damage in the hoof before changes in radiographic detail occur. When used at onset, the venogram proves to be most valuable. They can not only give baseline measurements but can also show the level of damage that has already occurred. Initial venograms allow farriers and veterinarians to evaluate what degree of corrective treatment is necessary for the best outcome. In addition, a series of venograms can show the process of pathology as well as recovery. From here, treatment can be altered to accommodate the case.

> *Key Words: laminitis* • *radiographs* • *treatment* • *venogram* • *hoof*



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HORSES AND THE ENVIRONMENT

DEVELOPMENT OF AN EQUINE BEST MANAGEMENT PRACTICE DEMONSTRATION WORKING HORSE FARM

C.A. WILLIAMS, M. WESTENDORF, C. OBROPTA, S. RALSTON, W. BAMKA and K.. MALINOWSKI

The goal of this project was to create a research and educational venue at the equine research facility of the Rutgers New Jersey Agriculture Experiment Station, under the auspices of the Equine Science Center (ESC). The overall goal was to showcase best management practices (BMPs) for equine and other animal facilities. This goal was met by accomplishing the following objectives: 1) implementation of stormwater BMPs that will minimize water quality impacts and evaluating the efficacy of these practices, 2) establishment of pasture management strategies that are protective of the environment while maximizing forage quality and availability, 3) creating an effective manure management system to minimize the environmental impacts of animal waste, and 4) conducting educational demonstrations and workshops showcasing the BMPs for stakeholders.



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DIETARY PROTEIN AFFECTS NITROGEN AND Ammonia Excretion in Horses

C.A. WILLIAMS, C. URBAN and M.L. WESTENDORF

Adding excess protein to the diet of horses and other animals leads to the excess being excreted in the sweat, feces, and urine as nitrogen, as well as possible effects on performance. It has been shown that horse owners commonly overfeed protein [1]. Higher amounts of nitrogen in the feces and urine must be managed in order to prevent consequences to the environment, such as water contamination and decreased air quality [2]. Ammonia contamination has the potential for at least short-term adverse effects on agricultural workers involved in animal care [3].



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EFFECTS OF BEDDING TYPE ON COMPOST QUALITY OF EQUINE STALL WASTE: IMPLICATIONS FOR SMALL HORSE FARMS

S. KOMAR, R. MISKEWITZ, M. WESTENDORF and C. A. WILLIAMS

Our objective in this study is to compare 4 of the most common bedding materials used by equine operations on the chemical and physical characteristics of composted equine stall waste. Twelve Standardbred horses were adapted to the barn and surrounding environment for 2 wk before the start of the study. Groups of 3 horses were bedded on 1 of 4 different bedding types (wood shavings, pelletized wood materials, long straw, and pelletized straw) for 16 h per day for 18 d. Stalls were cleaned by trained staff daily, and all contents removed were weighed and stored separately by bedding material on a level covered concrete pad for the duration of the study. Compost piles were constructed using 3 replicate piles of each bedding type in a randomized complete block design. Each pile was equipped with a temperature sensor and data logger. Water was added and piles were turned weekly throughout the 100-d compost process. Initial and final samples were taken, dried, and analyzed for DM mass, OM, inorganic nitrogen (nitrate-N and ammonium-N), electrical conductivity, and soluble (plant-available) nutrients. Data were analyzed using the GLM procedure, and means were separated using Fischer's protected LSD test (P<0.05). No significant temperature differences were observed among the bedding materials. The composting process resulted in significant reductions (P<0.05) in DM mass for each of the 4 bedding materials. The composting process resulted in significant reductions (P<0.05) in OM and C:N ratio for all 4 bedding materials. The composted long straw material had greater concentrations of total Kjeldahl nitrogen (P<0.05), nitrate-N (P<0.05), and ammonium-N (P<0.05) than the composted wood shavings. This study demonstrated that incorporating a simple aerobic composting system may greatly reduce the overall volume of manure and yield a material that is beneficial for land application in pasture-based systems. The straw-based materials may be better suited for composting and subsequent land application; however, factors such as suitability of the bedding material for equine use, material cost, labor, and availability must be considered when selecting a bedding material.

> *Key Words: bedding* • *compost* • *equine* • *manure* • *nitrogen* • *stall waste*



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HORSES AND THE ENVIRONMENT

ENVIRONMENTAL IMPACTS OF EQUINE OPERATIONS: A U.S. DEPARTMENT OF AGRICULTURE MULTISTATE PROJECT

M.L. WESTENDORF, C.WILLIAMS, A.O. BURK, N. TROTTIER, K. MARTINSON, P.D. SICILIANO, A.M. SWINKER, E.A. GREENE and R. BOTT

The U.S. Department of Agriculture supports agricultural research by encouraging the formation of multidisciplinary and multi-institutional teams. Project teams focus on agricultural issues related to profitability and economic and environmental sustainability. Recently, a U.S. Department of Agriculture project to study the impact of equine management and feeding practices on the environment was approved. The project, "NE-1041: Environmental Impacts of Equine Operations," is a Northeast regional project but includes research and extension faculty from across the country. The project team includes representatives from Alabama, Connecticut, Kentucky, Louisiana, Maryland, Michigan, Minnesota, New Jersey, North Carolina, Pennsylvania, South Dakota, and Vermont. The goal of this project is to incorporate the best available data on horse management and feeding practices, manure storage and disposal, pasture and cropping management, soil and environmental quality, erosion control, and farm management practices to minimize negative impacts of equine operations on the environment. The specific objectives of the project are to assess existing data on environmental impacts of equine operations, identify gaps in current knowledge, conduct research when data are lacking or nonexistent, and incorporate existing and newly generated data into a systematic description of nutrient flow in soil, water, and air occurring on horse farms. Estimates will be made of pathogen transports and nitrogen (N)-, phosphorus (P)-, potassium (K)-, and energy (carbon)-loss potentials. In addition to identifying systemwide losses on equine farms, another goal of the project is to assist farmers and agricultural professionals in determining the value of equine management practices and other accepted best management practices.

> Key Words: equine • manure management • environmental quality • pasture management • BMP



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CASE STUDY: MANURE MANAGEMENT PRACTICES ON NEW JERSEY EQUINE FARMS

M.L. WESTENDORF, T. JOSHUA, S.J. KOMAR, C. WILLIAMS and R. GOVINDASAMY

The number of equine farms is increasing in Northeast and Mid-Atlantic states. These farms may influence environmental and water quality because of manure collection, storage, spreading, and disposal practices. A manure management survey was mailed to 2,000 New Jersey equine farms during the winter of 2006 to 2007. Of the 2,000 surveys sent, 472 were returned; 18.5% were from training or performance farms, and the remainder (81.5%) were from breeding, boarding, or pleasure farms. Fifty-four percent of all farms spread manure on their farms. Of those who spread manure, only 27% had more than 8.09 ha (20 acres) available for spreading. Seventy-four percent had a designated area for manure storage. Eighty-three percent said their manure storage was greater than 61 m (200 ft) from water or wetlands, and 86% said storage was greater than 61 m (200 ft) from neighbors. Data were modeled to determine the relationship between manure storage or manure spreading and other management practices. The storage model showed that farms with 6 to 10 horses were more likely to have storage facilities than farms not included in the model. This model had a predictive accuracy of 83.3% and an R2 of 0.35 (P < 0.01). The manure spreading model showed that those who spread manure were also likely to credit manure for its fertilizer value. The spreading model had an overall predictive accuracy of 95.5% and an R2 of 0.795 (P < 0.01). These results indicate that although most equine farms did not pose a direct risk to water quality or to a neighbor, most do not currently use best management practices in managing, spreading, or storing manure.

> Key Words: equine • manure management • water quality • manure storage • manure spreading



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HORSES AND THE ENVIRONMENT

PRODUCTION AND ENVIRONMENTAL IMPLICATIONS OF EQUINE GRAZING

R.C. BOTT, E.A. GREENE, K. KOCH, K.L. MARTINSON, P.D. SICILIANO, C. WILLIAMS, N.L. TROTTIER, A. BURK and A. SWINKER

Horses' physical and digestive well-being is often enhanced when allowed to graze on pastures. Furthermore, a well-managed pasture can contribute to economic viability. Grazing can however have deleterious effects on the environment if not properly managed. Although equine grazing, defecating, and ground trampling behavior is unique from that of other livestock species, pasture management practices are often based on those derived from cattle grazing. This review summarizes the current knowledge of impacts of equine grazing on pasture quality and environment and identifies gaps where further information is needed to formulate and recommend sustainable grazing methods specific to equine.

> *Key Words: environment* • *equine* • *grazing* • *pasture*



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HUMAN EXERCISE PHYSIOLOGY

CAFFEINE VS CAFFEINE-FREE SPORTS DRINKS: EFFECTS ON URINE PRODUCTION AT REST AND DURING PROLONGED EXERCISE

R.D. WEMPLE, D.R. LAMB and K.H. McKEEVER

We compared the effects of caffeinated vs non-caffeinated carbohydrate electrolyte (CE) drinks on urine volume (UV), free water clearance (C_{H20}), fractional excretion of water (FE_{H20}), and osmolar excretion during 4 h of rest or 1 h rest followed by 3 h of cycling at 60 % V0_{2max} in six subjects. We also tested maximal performance at 85 % V0_{max} following the 3-h exercise trials. Throughout the two resting trials and the two rest + exercise trials, subjects ingested CE (total volume = 35 ml/kg) without (PLAC) or with (CAFF) caffeine (25 mg/dl). Blood samples were collected, and body weight and UV were recorded every hour. Urine and blood were analyzed for osmolality and creatinine, and plasma catecholamine concentrations were determined. At rest, mean (± SE) UV between 60 min and 240 min was greater for CAFF (1843 ± 166 ml) vs PLAC (1411 ± 181 ml) (P<0.01); during exercise the difference in UV between CAFF (398 \pm 32 ml) and PLAC (490 \pm 57 ml) was not significant. Cycling performance was unaffected by caffeine. Plasma catecholamine concentrations were not different between PLAC and CAFF but were greater during exercise than rest (P<0.01) and may have counteracted the diuretic effect of caffeine observed at rest. Thus, CAFF consumed in CE during moderate endurance exercise apparently does not compromise bodily hydration status.

> *Key Words: caffeine* • *endurance exercise* • *renal function*



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THE EFFECTS OF A POST-WORKOUT NUTRACEUTICAL DRINK ON BODY COMPOSITION, PERFORMANCE AND HORMONAL AND BIOCHEMICAL RESPONSES IN DIVISION I COLLEGE FOOTBALL PLAYERS

S.M. ARENT, P. DAVITT, D.L. GOLEM, C.A. WILLIAMS, K.H. MCKEEVER and C. JAOUHARI

Football players walk a fine line between optimal training and overtraining. Manipulating nutrient intake has the potential to maximize the biochemical environment necessary to induce peak performance and proper recovery. The purpose of this study was to examine the impact of supplementing the diet of Division I football players with a proprietary nutraceutical recovery drink on changes in performance, body composition, anabolic status, muscle damage, inflammation and oxidative stress over the course of a 7-week conditioning period immediately prior to preseason camp. At the beginning (trial 1) and end (trial 2) of a 7-week training phase, body composition, vertical jump and 225 lb bench press were assessed in Division I college football players (n=25). A 30 s Wingate Anaerobic Test plus eight 10 s intervals was used to examine power and biochemical responses. Blood samples were collected pre-, 0 and 60 min post-test for analysis of interleukin-6 (IL), 8-isoprostane (8-iso), cortisol (CORT) and resting testosterone:CORT (T:C) ratios. Athletes were randomly assigned to either an experimental group (EXP) receiving the nutraceutical drink (n=13) or a control group (CON) receiving an isocaloric equivalent (n=12). EXP had a significantly greater increase in peak power (P<0.05) and significant decreases in percentage body fat and fat mass (P<0.05). Multivariate ANOVA for repeated measures (RM MANOVA) revealed a significant test £ time £ group interaction (P<0.05) for changes in CORT, IL-6 and 8-iso from trial 1 to trial 2. Follow-ups revealed no significant differences between groups at trial 1 for any of the variables. At trial 2, EXP had significantly lower CORT at rest (P=0.01) and 60 min post-test (P=0.001). Additionally, IL-6 was significantly different between EXP and CON at 0 (P<0.01) and 60 min post-test (P<0.01), with CON having an elevated IL-6 response. There were also differences in both 8-iso and creatine kinase at all time points at trial 2, with CON having higher levels (P<0.02.). There were significant differences between groups in T:C ratio changes (P<0.05), with EXP having an improved T:C ratio. It appears that supplementing the post-workout diet of Division I college football players with a nutraceutical recovery drink has favorable effects on body composition, peak power output and biochemical markers. Based on differences between groups that emerged at rest at trial 2, it appears that this supplement positively impacts both acute and chronic physiological responses indicative of improved recovery.

Key Words:

antioxidant • ergogenic aids • anaerobic power • oxidative stress • hypothalamic-pituitary-adrenal axis • superoxide dismutase



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HUMAN EXERCISE PHYSIOLOGY

THE EFFECTS OF THEAFLAVIN-ENRICHED BLACK TEA EXTRACT ON MUSCLE SORENESS, OXIDATIVE STRESS, INFLAMMATION, AND ENDOCRINE RESPONSES TO ACUTE ANAEROBIC INTERVAL TRAINING: A RANDOMIZED, DOUBLE-BLIND, CROSSOVER STUDY

S.M. ARENT, M. SENSO, D.L. GOLEM and K.H. McKEEVER

Background: Muscle soreness and decreased performance often follow a bout of highintensity exercise. By reducing these effects, an athlete can train more frequently and increase long-term performance. The purpose of this study is to examine whether a highpotency, black tea extract (BTE) alters the delayed onset muscle soreness (DOMS), oxidative stress, inflammation, and cortisol (CORT) responses to high-intensity anaerobic exercise.

Methods: College-age males (N=18) with 1+ yrs of weight training experience completed a double-blind, placebo-controlled, crossover study. Subjects consumed the BTE (1,760 mg BTE·d-1) or placebo (PLA) for 9 days. Each subject completed two testing sessions (T1 & T2), which occurred on day 7 of the intervention. T1 & T2 consisted of a 30 s Wingate Test plus eight 10 s intervals. Blood samples were obtained before, 0, 30 & 60 min following the interval sessions and were used to analyze the total to oxidized glutathione ratio (GSH:GSSG), 8- isoprostane (8-iso), CORT, and interleukin 6 (IL-6) secretion. DOMS was recorded at 24 & 48 h post-test using a visual analog scale while BTE or PLA continued to be administered. Significance was set at P<0.05.

Results: Compared to PLA, BTE produced significantly higher average peak power (P=0.013) and higher average mean power (P=0.067) across nine WAnT intervals. BTE produced significantly lower DOMS compared to PLA at 24 h post test (P<0.001) and 48 h post test (P<0.001). Compared to PLA, BTE had a slightly higher GSH:GSSG ratio at baseline which became significantly higher at 30 and 60 min post test (P<0.002). AUC analysis revealed BTE to elicit significantly lower GSSG secretion (P=0.009), significantly higher GSH:GSSG ratio (P=0.001), and lower CORT secretion (P=0.078) than PLA. AUC analysis did not reveal a significant difference in total IL-6 response (P=0.145) between conditions.

Conclusions: Consumption of theaflavin-enriched black tea extract led to improved recovery and a reduction in oxidative stress and DOMS responses to acute anaerobic intervals. An improved rate of recovery can benefit all individuals engaging in high intensity, anaerobic exercise as it facilitates increased frequency of exercise.



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HUMAN EXERCISE PHYSIOLOGY

NUTRITIONAL SUPPLEMENTATION, PERFORMANCE, AND OXIDATIVE STRESS IN COLLEGE SOCCER PLAYERS

S.M. ARENT, J.K. PELLEGRINO, C.A. WILLIAMS, D.A. DIFABIO and J.C. GREENWOOD

The purpose of this study was to examine changes in performance and metabolic parameters in collegiate soccer players during preseason preparation and to determine the impact of a nutraceutical blend proposed to reduce oxidative stress. Male Division I college soccer players (n=22) performed a progressive maximal treadmill test at the beginning and end of preseason to assess changes in VO_{2max}, velocity at lactate threshold (V_{LT}), time-to-exhaustion, lipid hydroperoxide (LPO), 8-isoprostane, and creatine kinase (CK) response. After baseline testing, athletes were randomly assigned to receive the nutraceutical blend (EXP; n=12) or an isocaloric equivalent (CON; n=10) for 20 days of preseason training. ΔVO_{2max} (2.1 ± 3.3 ml/kg/min, P=0.007), DVLT (0.8 ± 1.4 km/h, P=0.045), and Δ time-to-exhaustion (39.4 ± 77.4 seconds, P=0.033) were improved across groups, but a significant effect of supplementation on performance was not seen. Changes in resting levels of CK from the beginning to end of preseason were significantly lower (P=0.044) in EXP (64.8 ± 188.4 U/L) than in CON (292.8 ± 304.8 U/L). Additionally, EXP demonstrated a significant decrease in the magnitude of the 8-isoprostane response at Trial 2 compared with Trial 1 (effect size [ES] = 20.74), whereas CON had an increased response (ES = 0.20). A similar pattern was seen for LPO (P=0.067). Preseason training in male college soccer players resulted in significant improvements in VO_{2max}, VLT, and time-to-exhaustion. Supplementing with a proprietary antioxidant and nutraceutical blend may enhance some of these effects as indicated by magnitude of the responses. However, it appears that the most notable effects of supplementation were seen for reduced CK and oxidative stress, at least with short-term supplementation.

> *Key Words: antioxidant* • *lactate threshold* • 8-*isoprostane* • *reactive oxygen species* • *superoxide dismutase*



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ACUTE EFFECTS OF SHORT-TERM FEED DEPRIVATION AND REFEEDING ON CIRCULATING CONCENTRATIONS OF METABOLITES, INSULIN-LIKE GROWTH FACTOR I, INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS, SOMATOTROPIN, AND THYROID HORMONES IN ADULT GELDINGS.

R.A. CHRISTENSEN, K. MALINOWSKI, A.M. MASSENZIO, H.D. HAFS and C.G. SCANES

Two studies were performed with Standardbred geldings 7 to 21 yr of age to determine the sequence of changes in blood plasma concentrations of some hormones and metabolites during feed deprivation for 48 h and for 12 h after refeeding. Plasma hormone and metabolite concentrations were determined with methods validated for horse plasma. Insulin-like growth factor binding proteins (IGFBP) were determined with radioligand analysis following SDS-PAGE electrophoresis. In both experiments, plasma concentrations of triiodothyronine and thyroxine decreased (P<0.01) during feed deprivation and increased (P<0.01) during refeeding. Plasma glucose and IGF-I either decreased or were not altered during feed deprivation. In contrast, plasma concentrations of NEFA and urea nitrogen increased (P<0.01) during feed deprivation and decreased (P<0.01) during the refeeding period. Plasma somatotropin (ST) increased (P<0.01) approximately 80% at 24 to 36 h of feed deprivation, declined (P<0.01) to control values at 48 h of feed deprivation, increased (P<0.01) nearly three fold at 3 h after refeeding, and returned to control values by 6 h after refeeding. We identified five IGFBP, and their plasma concentrations were not significantly altered during feed deprivation or following refeeding. We conclude that metabolite availability during feed deprivation and following refeeding alters the secretion of thyroid hormones, ST, and possibly IGF-I, thereby maintaining homeostasis in horses.

Key Words: horses • *food deprivation* • *somatotropin* • *insulin-like growth factor*



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DISTRIBUTION OF GLUTAMINE SYNTHETASE AND AN INVERSE RELATIONSHIP BETWEEN GLUTAMINE SYNTHETASE EXPRESSION AND INTRAMUSCULAR GLUTAMINE CONCENTRATION IN THE HORSE

H.C. MANSO FILHO, H.E.C. COSTA, Y. WANG, K.H. McKEEVER and M. WATFORD

Glutamine plays important roles in the interorgan transport of nitrogen, carbon and energy but little is known about glutamine metabolism in the horse. In this study we determined the tissue distribution of glutamine synthetase expression in three Standardbred mares. Expression of glutamine synthetase was highest in kidney and mammary gland, and relatively high in liver and adipose tissue. Expression was lower in gluteus muscle, thymus, colon and lung, and much lower in small intestine, pancreas and uterus. The pattern of glutamine synthetase expression in the horse is similar to that of other herbivores and it is likely that skeletal muscle, liver, adipose tissue and lungs are the major sites of net glutamine synthesis in this species. Expression did not differ between adipose tissue depots but did vary between different muscles. Expression was highest in gluteus and semimembranous muscles and much lower in diaphragm and heart muscles. The concentration of intramuscular free glutamine was inversely correlated with expression of glutamine synthetase (r=-0.81, P=0.0017). The concentration of free glutamine was much higher in heart muscle $(21.6 \pm 0.9 \,\mu\text{mol/g wet wt})$ than in gluteus muscle $(4.19 \pm 0.33 \,\mu\text{mol/g}$ wet wt), which may indicate novel functions and/or regulatory mechanisms for glutamine in the equine heart.

> *Key Words: equine* • *glutamine synthetase* • *horse* • *muscle*



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DIURNAL VARIATION OF GHRELIN, LEPTIN, AND ADIPONECTIN IN STANDARDBRED MARES

M.E. GORDON and K.H. McKEEVER

Twelve Standardbred mares underwent blood sampling for 24 h to test the hypothesis that there is diurnal variation of humoral mediators of peripheral energy balance including active ghrelin, adiponectin, leptin, glucose, insulin, and cortisol. The experiment was conducted under acclimated conditions. Grass hay and pelleted grain were provided at 0730 and 1530. Plasma concentrations of active ghrelin and leptin concentrations both peaked ($47.3 \pm 6.5 \text{ pg/mL}$ and $5.9 \pm 1.1 \text{ ng/mL}$, respectively; P<0.05) at 1550, 20 min after feeding. Active ghrelin decreased (P<0.05) to 28.9 ± 4.5 pg/mL overnight. The nadir of leptin $(4.6 \pm 0.9 \text{ ng/mL})$ occurred at 0650. Neither hormone showed variation (P>0.05) after the morning feeding. Plasma glucose and insulin concentrations increased (P<0.05) in response to feeding; however, the morning responses (glucose = $96.9 \pm 2.6 \text{ mg/dL}$; insulin = $40.6 \pm 7.3 \text{ uIU/mL}$) were greater (P<0.05) than the afternoon responses (glucose = $89.9 \pm 1.8 \text{ mg/dL}$; insulin = 23.2 ± 4.3 uIU/mL at 180 and 60 min after feeding, respectively). Cortisol concentrations increased (P < 0.05) during the morning hours, but did not respond to feeding, whereas adiponectin concentrations remained stable throughout the study. Hence, active ghrelin and leptin may be entrained to meal feeding in horses, whereas adiponectin seems unaffected. We concluded that there seems to be a diurnal variation in glucose and insulin response to a meal in horses. Furthermore, elevated glucose and insulin concentrations resulting from the morning feeding may be responsible for the increase in leptin concentration in the afternoon.

> *Key Words: adiponectin* • *diurnal variation* • *ghrelin* • *horse* • *leptin*



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THE EARLY INSULIN RESPONSE TO AN INTRAVENOUS GLUCOSE TOLERANCE TEST IN HORSES

A. GIRAUDET, K.W. HINCHCLIFF, C.W. KOHN and K.H. McKEEVER

Plasma insulin concentration of many species has a characteristic early or acute-phase response in the minutes after IV administration of glucose. However, the plasma insulin response of horses soon after the IV administration of glucose has not been examined, whereas the more prolonged response has been evaluated.

We examined the plasma insulin and glucose concentration responses of adult mares during the 30 minutes after rapid IV administration of glucose (0.33 g/kg of body weight).

Plasma glucose concentration peaked at 664 ± 54 mg/dl within 1 minute of cessation of glucose administration, whereas insulin concentration peaked at 326 ± 24 pmol/L at 2 minutes after the end of glucose administration. Thus, these mares had an acute insulin response, consistent with that observed in other species, including dogs, human beings, and cattle.



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INTERVAL EXERCISE ALTERS FEED INTAKE AS WELL AS LEPTIN AND GHRELIN CONCENTRATIONS IN STANDARDBRED MARES

M.E. GORDON, K.H. McKEEVER, S. BOKMAN, C.L. BETROS, H. MANSO-FILHO, N. LIBURT and J. STRELTSOVA

Reasons for performing study: Horses in training tend to become inappetant; however, the mechanism responsible for this training-induced inappetance is not known.

Hypothesis: Training and/or ulcers alter the feed intake (FI) and hormonal and/or biochemical (active ghrelin, leptin, glucose, insulin and cortisol) responses to acute high intensity exercise.

Methods: Eight Standardbred mares underwent 3 interval exercise tests (IET) and 3 parallel control tests (CON) before (IET1) and after 8 weeks of training (IET2) and after treatment for gastric ulcers (IET3). Plasma samples were taken before (0 min), during (last 10 sec of velocities eliciting 40, 100, and 20% VO_{2max}), and after (30 min, 60 min, 24 h) exercise (EX) or CON tests for RIA and colorimetric measurement of the concentrations of the above parameters. Samples were also collected before and after feeding. Horses were trained at a work intensity of 70% HR_{max} for 30 min/day, 5 days per week with FI measured daily.

Results: There were no changes (P>0.05) in any variable during the parallel control trials. However, there was a mismatch between FI and digestible energy (DE) requirements (P<0.05) with EX horses not meeting their DE requirements during the post training IETs. During all IETs, ghrelin, glucose and cortisol increased (P<0.05) during EX. Leptin only increased (P<0.05) during EX in the post training IETs. Insulin remained low during EX, but increased (P<0.05) post EX.

Conclusion: High intensity exercise appeared to be associated with decreases in FI and alterations of leptin and ghrelin.

Potential relevance: More research is needed to determine if there is a relationship between alterations of these hormones and changes in FI in horses that lose weight while in training.

Key Words: horse • *energy homeostasis* • *appetite* • *endocrine* • *training*



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NOVEL FINDINGS REGARDING GLUT-4 EXPRESSION IN ADIPOSE TISSUE AND MUSCLE IN HORSES – A PRELIMINARY REPORT

H.C. MANSO FILHO, K.H. McKEEVER, M.E. GORDON, H.E.C. COSTA and M. WATFORD

One of the hallmarks of insulin resistance is a reduction in glucose transporter-4 (Glut-4) expression in adipose tissue but not in skeletal muscle. However, while Glut-4 has been demonstrated in skeletal and cardiac muscles in horses it has not been demonstrated in adipose tissue. The initial objectives of the present study were: (1) to test the hypothesis that Glut-4 expression would vary between selected key skeletal muscles; (2) to test the hypothesis that it would also vary between representative adipose tissue depots, and (3) to see whether expression would be greater in adipose tissue compared to muscle. Glut-4 expression was determined by Western blot using samples obtained from post mortem biopsies obtained from four muscles (gluteus medius, semitendinosus, heart, and diaphragm), and four adipose tissues (subcutaneous, retroperitoneal, mesenteric, and omental) in three horses. There were no differences (P>0.05) in Glut-4 protein expression between the muscles sampled. Likewise there were no differences (P>0.05) in Glut-4 protein expression between fat depots. There was a significant difference (P=0.03) when pooled means for Glut-4 expression in muscle (58.8 ± 2.5 densitometry units) were compared with adipose tissue (115.8 \pm 15.7). This difference in Glut-4 expression in these two tissues with distinctly different metabolic reasons for taking up glucose may warrant further investigation to see if there are more pronounced differences in Glut-4 expression in muscle and adipose tissue in various populations of horses.

Key Words: equine • *glucose metabolism* • *insulin sensitivity* • *body composition*



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ORAL AND INTRAVENOUS CARBOHYDRATE CHALLENGES DECREASE ACTIVE GHRELIN CONCENTRATIONS AND ALTER HORMONES RELATED TO CONTROL OF ENERGY METABOLISM IN HORSES

M.E. GORDON and K.H. McKEEVER

This study tested the hypothesis that grain and intravenous dextrose challenges would alter plasma concentrations of active ghrelin, adiponectin, leptin, glucose, insulin, and cortisol in Standardbred mares. To deliver 0.5 g of glucose (dextrose solution for the intravenous test)/kg of BW, mares received intravenous dextrose (50% solution) or oral grain administration in 2 trials. In response to the oral grain challenge, plasma glucose and insulin concentrations increased (P<0.001) by 56 and 802%, respectively. Plasma ghrelin concentration initially decreased (P<0.001) by 40%, then subsequently increased (P<0.05) from its nadir by 259%. Plasma leptin concentration decreased (P=0.002) 17% compared with baseline. There was no change (P=0.34) in plasma adiponectin concentration in response to oral grain challenge; however, plasma cortisol concentrations decreased (P<0.001) by 24%. In response to the intravenous dextrose challenge, plasma glucose and insulin concentrations increased (P<0.001) by 432 and 395%, respectively. Plasma active ghrelin concentration initially decreased (P<0.001) by 56%, then subsequently increased (P<0.001) from its nadir by 314%. Plasma leptin concentration also increased (P<0.001) by 33% compared with baseline. There was no change (P=0.18) in plasma adiponectin concentration throughout the dextrose challenge. Plasma cortisol concentration increased (P=0.027) by 20%. Hence, oral grain and intravenous nutrient challenges have the ability to alter variables potentially related to energy metabolism in mares, with acute changes in glucose and insulin possibly modulating changes in ghrelin and leptin.

> Key Words: adiponectin • ghrelin • horse • leptin



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PLASMA GLUCOSE AND INSULIN RESPONSES OF Thoroughbred mares fed a meal high in starch and sugar or fat and fiber

C.A. WILLIAMS, D.S. KRONFELD, W.B. STANIAR and P.A. HARRIS

Plasma concentrations of glucose and insulin following a meal were compared in twelve Thoroughbred mares fed a pelleted concentrate (PC), a traditional sweet feed high in sugar and starch (SS), or a feed high in fat and fiber (FF). The feeds had similar DE and CP but differed in fat (19, 32, and 166 g/kg DM, respectively), NDF (199, 185, and 369 g/kg DM, respectively) and nonstructural carbohydrates (574, 645, and 247 g/kg, respectively). Mares were randomly assigned to two groups balanced for foaling date and weight. All mares received PC in late gestation; then, after foaling, one group was fed SS and the other FF for trials in early and late lactation. Mares were placed in stalls and deprived of feed overnight. A series of blood samples was collected via a jugular catheter from 0 (baseline) to 390 min after consumption of 1.82 kg of feed. Plasma was analyzed for glucose and insulin. Baseline values, peak values, and areas under curves (AUC) were compared by ANOVA. Baseline values were $74.7 \pm 10.9 \text{ mg/dL}$ for glucose and 5.86 \pm 1.80 mIU/L for insulin for all diets and stages. Responses to PC did not differ between the two groups (P>0.34), indicating the groups were metabolically similar. Peak plasma glucose and insulin concentrations were higher (P<0.001) in the SS group than in the FF group during early and late lactation. Similarly, glucose and insulin AUC were larger (P<0.003) in SS than in FF during early and late lactation. These results indicate that metabolic fluctuations are moderated by the replacement of sugar and starch with fat and fiber. This replacement may reduce the risk of certain digestive and metabolic disorders that have been linked to feeding meals of grain-based concentrates to pregnant or lactating mares.

> Key Words: dietary fat • fiber • glucose • horses • insulin



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PLASMA LEPTIN, GHRELIN AND ADIPONECTIN CONCENTRATIONS IN YOUNG FIT RACEHORSES VERSUS MATURE UNFIT STANDARDBREDS

M.E. GORDON, K.H. McKEEVER, C.L. BETROS and H.C. MANSO FILHO

Concentrations of hormones related to energy homeostasis may differ between populations with varied body compositions, acting as signals to increase or decrease energy intake and/or expenditure. How these parameters correlate with body composition in horses and how they vary in fit (F) versus unfit (UF) Standardbred racehorses is unclear. The purpose of this study was to test the hypothesis that plasma concentrations of glucose (GLU), insulin (INS), cortisol (CORT), ghrelin (GHRL), adiponectin (ADIP) and leptin (LEP) would be correlated with body composition and differ in fit (F) versus unfit (UF) horses. Fasting plasma samples were taken from 12 unfit (11 ± 2 years, 521 ± 77 kg; mean \pm SD) and 34 fit (4 ± 2 years, 475 ± 83 kg) Standardbred horses. GHRL, LEP, ADIP, INS and CORT concentrations were measured using radioimmunoassay. GLU concentration was measured using colorometric kits. Body composition data included body weight, body condition score (BCS), and percent fat (%fat) calculated using rump fat thickness measured ultrasonically and the Westervelt equation. Data were analyzed using Pearson Product moment and Student's *t* tests.

There were no differences (P>0.05) between F and UF horses for the plasma concentrations of CORT (69 ± 14 versus 76 ± 23 lg/dL), INS (7.2 ± 3.5 versus 7.1 ± 1.8 IIU/mL) or GLU (90 ± 6 versus 86 ± 7 mg/dL). Plasma GHRL and ADIP concentrations were greater (P<0.05) in F versus UF horses (54 ± 27 versus 33 ± 17 pg/mL and 1820 ± 276 versus 1333 ± 249 ng/mL, respectively), while plasma LEP was lower in F versus UF (1.0 ± 0.6 versus 4.4 ± 2.4 ng/mL, P<0.001). BCS and %fat were lower in F versus UF horses (4.8 ± 0.3 versus 6.7 ± 0.5 and 11.9 ± 1.6 versus 15.4 ± 2.5%, respectively), with no correlation between %fat and GHRL (-0.12, P>0.05), although there was a positive correlation between %fat and LEP (+0.72, P<0.05), and a negative correlation between %fat and LEP (+0.72, P<0.05), and a negative correlation between %fat and LEP (+0.72, P<0.05), and a negative correlation between %fat and LEP (+0.72, P<0.05), and a negative correlation between %fat and LEP (+0.72, P<0.05), and a negative correlation between %fat and LEP (+0.72, P<0.05), and a negative correlation between %fat and LEP (+0.72, P<0.05), and a negative correlation between %fat and LEP (+0.72, P<0.05), and a negative correlation between %fat and LEP (+0.72, P<0.05), and a negative correlation between %fat and ADIP (-0.40, P<0.05). The data show that in comparing fit and unfit horses, there are variations in body composition as well as concurrent and substantial differences in the concentrations of hormones, cytokines, and other parameters related to the control of appetite and feed intake.

Key Words: adipocytokines • *ghrelin* • *body composition* • *horse*



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NUTRACEUTICALS

ANTIOXIDANT SUPPLEMENTATION AND SUBSEQUENT OXIDATIVE STRESS OF HORSES DURING AN 80-KM ENDURANCE RACE

C.A. WILLIAMS, D.S. KRONFELD, T.M. HESS, K.E. SAKER, J.N. WALDRON, K.M. CRANDELL, R.M. HOFFMAN and P. A. HARRIS

This study tested the development of oxidative stress and the effects of antioxidant supplementation in an 80-km ride. A pre-competition survey revealed that no competitor would participate without vitamin E supplementation; therefore, 46 horses were paired for past performances and randomly assigned to two groups of 23 each for 3 wk of supplementation before the ride. One group (E) was orally supplemented with 5,000 IU of vitamin E per day; the other group (E+C) received that dose of vitamin E plus 7 g/d of vitamin C. Blood samples, temperature, and heart rate were taken the day before the race, at 21 and 56 km during the ride, at completion, and after 20 min of recovery. Plasma was assayed for lipid hydroperoxides, α-tocopherol, total ascorbate, albumin, creatine kinase (CK), and aspartate aminotransferase (AST). Total glutathione and glutathione peroxidase activity were determined in red blood cells and white blood cells. Thirty-four horses completed the race, 12 horses (six in E and six in E+C) did not finish for reasons including lameness, metabolic problems, and rider option. Plasma ascorbate was higher (P=0.045) in the E+C group than in the E group. Other than ascorbate, neither antioxidant status nor CK and AST activities were affected by supplementation with E+C vs. E. Red blood cell glutathione peroxidase, white blood cell total glutathione, lipid hydroperoxides, CK, and AST increased, and red blood cell total glutathione and white blood cell glutathione peroxidase activity decreased with distance (P<0.001). Positive correlations were found for plasma lipid hydroperoxides on CK (r=0.25; P=0.001) and AST (r=0.33; P<0.001). These results establish an association between muscle leakage and a cumulative index of oxidative stress.

> Key Words: α-Tocopherol • ascorbate • endurance exercise • equine • muscle enzymes • oxidative stress

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NUTRACEUTICALS

EFFECT OF ORANGE PEEL AND BLACK TEA EXTRACTS ON MARKERS OF PERFORMANCE AND CYTOKINE MARKERS OF INFLAMMATION IN HORSES

J.M. STRELTSOVA, K.H. McKEEVER, N.R. LIBURT, M.E. GORDON, H. MANSO FILHO, D.W. HOROHOV, R.T. ROSEN and W. FRANKE

This study tested the hypothesis that orange peel (O) and decaffeinated black tea (T) extracts would alter markers of exercise performance as well as exercise-induced mRNA expression for the inflammatory cytokines IL-6, TNF-alpha and IFN-gamma. Nine healthy, unfit Standardbred mares (age: 10 ± 4 years, 450kg) were assigned to three treatment groups in a randomized crossover design where each horse was administered one of the following; placebo (O; 21 water), black tea extract in water (T; 21) or orange peel extract in water (W; 21), via a nasogastric tube. One hour later the horses completed an incremental graded exercise test (GXT) on a treadmill at a fixed 6% grade with measurements and blood samples obtained at rest, at the end of each 1min step of the GXT and at 2 and 5min post-GXT. An additional set of blood samples for Polymerase Chain Reaction (PCR) measurements of mRNA was obtained before exercise and at 5 and 30 min and 1, 2, 4 and 24 h post-GXT. The GXTs were conducted between 0700 and 1200h not less than 7 days apart. There were no differences (P>0.05) in VO_{2max}, respiratory exchange ratio, run time, velocity at VO_{2max}, core body temperature, haematocrit, creatine kinase (CK), plasma lactate concentrations, HR, right ventricular pressure (RVP) or pulmonary artery pressure (PAP) across treatments. A major finding was that orange peel extract significantly reduced post-exercise VO₂ recovery time $(W=112 \pm 7, O=86 \pm 6, and T=120 \pm 11s)$. There was a significant difference in plasma total protein concentration (TP) in the O runs compared with water and T. TNF-alpha mRNA expression was lower in the T runs compared with water and O trials. IFNgamma mRNA expression levels appeared to be lower in both the T and O extract runs compared with the water trials. The mRNA expression of IL-6 was unaltered across treatment groups. These data suggest that orange peel and black tea extracts may modulate the cytokine responses to intense exercise. Orange peel extract reduced postexercise recovery time and may potentially enhance the ability of horses to perform subsequent bouts of high-intensity exercise.

Key Words:

equine • exercise • nutraceutical • tumor necrosis factor-alpha • interferon-gamma



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NUTRACEUTICALS

EFFECTS OF GINGER AND CRANBERRY EXTRACTS ON THE PHYSIOLOGICAL RESPONSE TO EXERCISE AND MARKERS OF INFLAMMATION IN HORSES

N.R. LIBURT, K.H. McKEEVER, J.M. STRELTSOVA, W.C. FRANKE, M.E. GORDON, H.C. MANSO FILHO, D.W. HOROHOV, R.T. ROSEN, C.T. HO, A.P. SINGH and N. VORSA

This study hypothesized that ginger (Zingiber officinale) and cranberry (Vaccinium macrocarpon) extracts would alter the physiological response to exercise as well as markers of muscle damage, and mRNA expression for the inflammatory cytokines tumour necrosis factor- α (TNF- α), interferon-g (IFN-g) and interleukin-6 (IL-6) after an exhaustive bout of exercise in horses. Nine unfit Standardbred mares (age 10 ± 4 years, \sim 450 kg) completed three graded exercise tests (GXTs) in a crossover design, where they were assigned to the initial order of treatment in a randomized fashion. The GXTs were conducted between 07.00 and 12.00 hours, 7 days apart. Mares received either water (21), cranberry (~30 g in 2 l of water) or ginger (~30 g in 2 l of water) extract 1 h prior to testing. Blood samples were taken prior to dosing (pre-exercise), at the end of each step of the GXT, at the end of the exercise and at 2, 5 and 30 min, 1, 2, 4 and 24 h post-GXT. Plasma total protein (TP) concentration and haematocrit (HCT) were analyzed immediately following the tests. Analysis of creatine kinase (CK) and aspartate aminotransferase (AST) as done commercially. There was no effect of treatment (P>0.05) on VO_{2max}, run-time to fatigue, core temperature, TP or HCT. CK was substantially elevated (P<0.05) in the ginger group at 4 h post-GXT. All CK levels returned to baseline 24 h post-GXT. No change (P>0.05) was noted in AST. A slight increase (P<0.05) in CK was seen in all groups at 2 h post-GXT. The cranberry group had significantly lower TNF-a mRNA expression than the control and ginger groups. Ginger appeared to influence (P<0.05) the upregulation and expression of IFN-g mRNA at 30 min post-GXT, but, more strikingly, significantly decreased recovery time defined as the time for VO₂ to recover from the peak observed at fatigue to a post-exercise plateau (ginger= 101 ± 3 s, water= 130 ± 14 s, cranberry= 131 ± 16 s). No effect of treatment or exercise (P>0.05) was seen on IL-6 mRNA expression. Results suggest that cranberry extract blunts the upregulation and expression of TNF-a mRNA, while ginger extract reduces cardiovascular recovery time in horses completing a short, exhaustive bout of exercise.

> Key Words: equine • exertion • flavonols • cytokines



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ORAL VITAMIN E SUPPLEMENTATION ON OXIDATIVE STRESS, VITAMIN AND ANTIOXIDANT STATUS IN INTENSELY EXERCISED HORSES

C.A. WILLIAMS and S.A. CARLUCCI

Reasons for performing study: Vitamin E is the most commonly supplemented antioxidant in horses; however, previous research is not conclusive as to the recommended level for exercising horses.

Objective: To evaluate the effects of 3 levels of vitamin E supplementation on oxidative stress and vitamin/antioxidant status in intensely exercised horses to determine the optimal level of vitamin E supplementation.

Methods: Twelve unfit Standardbreds were divided into 3 groups, supplemented orally with 0 (CON), 5000 (MOD), or 10,000 (HI) iu/day of DL-α-tocopheryl acetate. The 3 times 3 Latin square design consisted of three 4 week supplementation periods with 4 week wash out periods between. After each period, horses underwent a treadmill interval exercise test. Blood samples were collected and heart rate (HR) measured before, during and after exercise. Data were analyzed using ANOVA with repeated measures in SAS.

Results: The CON group had lower HR throughout the test compared to the MOD and HI groups (P<0.05). There was an increase in plasma retinol (RET), β -carotene (BC), red blood cell total glutathione and glutathione peroxidase with exercise (P<0.05), but all groups returned to baseline after 24 h. Plasma α -tocopherol (TOC) increased from baseline with exercise (P<0.0001) in all groups; treatment differences were observed at 24 h (P<0.05). The HI and CON groups had lower BC compared to the MOD group (P=0.05).

Conclusions: Horses supplemented with vitamin E, at nearly 10-times the 1989 NRC recommended level, did not experience lower oxidative stress compared to control horses. Additionally, lower plasma BC levels observed in the HI group, which may indicate that vitamin E has an inhibitory effect on BC metabolism.

Potential relevance: Supplementation above control levels is not more beneficial to oxidative stress and antioxidant status in intensely exercising horses; indeed, levels 10 times in excess may be detrimental to BC and should be avoided.

Key Words: horse • α-tocopherol • β-carotene • interval exercise • lipid hydroperoxides • retinol

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OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN INTENSELY EXERCISING HORSES ADMINISTERED NUTRACEUTICAL EXTRACTS

D.N. SMARSH, N. LIBURT, J. STRELTSOVA, K. McKEEVER and C.A. WILLIAMS

Reasons for performing study: Many nutraceuticals are used as equine supplements without their efficacy having been scientifically tested. Black tea, cranberries, orange peel and ginger are a few of those nutraceuticals that warrant further study.

Objective: To test the effects of single doses of black tea, cranberry, orange peel and ginger extract on markers of oxidative stress and antioxidant status following exercise in horses.

Methods: In Study 1, 9 mature, healthy but unfit Standardbred mares were administered 2 l of a control (water), orange peel extract (30 g extract) or decaffeinated black tea extract (28 g extract). In Study 2, the same mares were administered 2 l of a control (water), cranberry extract (30 g extract) or ginger extract (30 g extract). In each study, mares were given the extracts via nasogastric tube 1 h before performing a graded exercise test (GXT), in a randomized crossover design with at least 7 days between GXTs. Blood samples were collected at rest, at fatigue, and 1 and 24 h post exercise and analyzed for lipid hydroperoxides (LPO), total glutathione (GSH-T), glutathione peroxidase (GPx), α -tocopherol (TOC), β -carotene (BC) and retinol. Data were statistically analyzed using a repeated measures ANOVA.

Results: In Study 1, there was no effect of treatment for LPO, GSH-T, GPx, TOC or BC. Retinol was higher for both tea (P=0.0006) and water (P=0.004) than for orange peel. In Study 2, there was no treatment effect for LPO, GPx, GSH-T, RET, BC or TOC.

Conclusions: The results show that a single dose of various nutraceuticals in exercising horses do not produce an effect on either oxidative stress or antioxidant status and further investigation is needed as to whether long-term supplementation would enhance these effects.

Key Words:

horse • antioxidants • black tea • exercise • nutraceutical • orange peel



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Some commonly fed herbs and other functional foods in equine nutrition: A review

C.A. WILLIAMS and E.D. LAMPRECHT

Most herbs and functional foods have not been scientifically tested; this is especially true for the horse. This paper reviews some of the literature pertinent to herbal supplementation in horses and other species. Common supplements like Echinacea, garlic, ginger, ginseng, and yucca are not regulated, and few studies have investigated safe, efficacious doses. Ginseng has been found to exert an inhibitory effect on proinflammatory cytokines and cyclooxygenase-2 expression. Equine studies have tested the anti-inflammatory effects of a single dose of ginger, post-exercise. Echinacea has been reported to have anti-inflammatory and antioxidant properties. Yucca contains steroid-like saponins, which produce anti-inflammatory, antioxidant, and anti-spasmodic effects. However, some herbs have drug-like actions that interact with dietary components and marijuana. Horses fed garlic at >0.2 g/kg per day developed Heinz body anaemia. Drug-herb interactions are common and caution needs to be taken when implementing 'natural product' usage.

Key Words: anti-inflammatory • *herb* • *horse* • *immunostimulant* • *phytomedicine*



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VITAMIN E INTAKE AND SYSTEMIC ANTIOXIDANT STATUS IN COMPETITIVE ENDURANCE HORSES

C.A. WILLIAMS, D.S. KRONFELD, T.M. HESS, K.E. SAKER, J.E. WALDRON and P.A. HARRIS

The objective was to compare vitamin E content in the total diet of Arabian horses competing in the 80 km Research Ride 2002 to the oxidative stress and antioxidant measures throughout the race. Prior to the race, riders completed a detailed nutritional survey, which was used to calculate vitamin E intake. Blood samples, temperature and heart rate were taken the day before the race, at 27 and 48 km during the race, at race completion or elimination and 3 h after race completion. Plasma lipid hydroperoxides, a-tocopherol (TOC), creatine kinase (CK), aspartate aminotransferase (AST), albumin, red and white blood cell total glutathione and glutathione peroxidase were measured. Data were analyzed by Pearson's correlation in SAS. From the survey, it was estimated that horses consumed 2265 ± 114 IU/day of vitamin E. A negative correlation was found between the vitamin E intake and CK (r=-0.23; P=0.002), and AST (r=-0.22; P=0.003). A positive correlation was found between the vitamin E intake and plasma TOC (r=0.15; P=0.005) at all sample times. This suggests that intensely exercising horses supplemented with vitamin E may not have an improved welfare and performance if they are supplemented vitamin E versus what is naturally found in forages and commercial feeds.

Key Words: antioxidants • α-tocopherol • endurance • equine • muscle enzymes • nutritional survey



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NUTRITION

ABSORPTION OF DIFFERENT ORAL FORMULATIONS OF NATURAL VITAMIN E IN HORSES

N.M. FIORELLINO, E.D. LAMPRECHT and C.A. WILLIAMS

The objective of this study was to determine the effect of vitamin E source on plasma vitamin E concentration. Five different formulations of natural source vitamin E (4,000 IU/day) were supplemented to Standardbred mares (n=4 per group) for 14 days. Treatment 1 was given 10 g of 400 IU/g natural acetate (RRR- α -tocopheryl-acetate) powder, treatment 2 was given 6.66 g of 600 IU/g natural acetate powder, treatment 3 was given 6.66 g of 600 IU/g natural alcohol (RRR-a-tocopherol) powder, treatment 4 was given 20 g of 200 IU/g micellized natural alcohol powder, and treatment 5 was given 8mLof 500 IU/mL micellized natural alcohol liquid. Blood samples were collected before supplementation (day 0), after day 7 and day 14 of supplementation, and analyzed for plasma α-tocopherol. Treatments 1, 2, and 3 increased (P<0.05) from days 0 to 7, but remained similar at day 14. Treatments 4 and 5 also increased (P=0.004, and P<0.0001, respectively) from days 0 to 7 and were higher (P<0.05) than treatment 1, 2, and 3. Plasma levels peaked at day 7 and either plateaued or decreased (treatment 5, P=0.004) at day 14. The micellized alcohol formulations remained elevated (treatment 4) or decreased (treatment 5) after achieving peak blood concentration and, as hypothesized, tended to have plasma concentrations higher than the other vitamin E forms. Care should be taken to ensure that the most active biologically available form of vitamin E is provided in the diet to maximize its efficiency.

> *Key Words:* α-tocopherol • antioxidant • equine • natural • vitamin E



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NUTRITION

FAT ADAPTATION AFFECTS INSULIN SENSITIVITY AND ELIMINATION OF HORSES DURING AN 80 KM ENDURANCE RIDE

T.M. HESS, D.S. KRONFELD, K.H. TREIBER, K.E. CRANDELL, J.N. WALDRON, C.A. WILLIAMS, W.B. STANIAR, M.A. LOPES and P.A. HARRIS

The present study tested the hypothesis that insulin sensitivity would be lower in horses adapted to sugar and starch than those adapted to fat and fiber during an endurance race. Forty horses were divided into 3 dietary groups: one group of experimental feed (SS) was rich in starch (33%), low in fat (8%); another (FF) low in starch (6%) and rich in fat (15%); and a third group of commercial concentrates (CC) was intermediate in starch (16%) and fat (11%). Blood samples were taken the day before the race (PRE), within 3 minutes of arrival at each vet check (after 27, 48, 80 km), and three hours post completion or elimination. Plasma samples were analyzed for glucose, insulin, cortisol, glycerol, triglycerides, CK and AST. A proxy for insulin sensitivity as measured by the minimal model was calculated: RISQI=(1/sqrt [insulin]). Also, a proxy for pancreatic β -cell response to plasma glucose was calculated: MIRG =[800-0.30([insulin] -50)2]/[glucose -30]. Higher insulin in FF and CC horses, and in eliminated horses, combined with a lower RISQI in eliminated horses indicated that insulin resistance (low RISQI) was attenuated by fat and fiber feeding and decreased the likelihood of elimination. Higher insulin sensitivity in finishers and fat and fiber fed horses may have allowed a more efficient glucose uptake by muscles, allowing energy to be obtained through NEFA and TG. Fat and fiber feeding could avoid insulin resistance improving the efficiency of energy utilization and performance of horses during endurance races it also could reduce excitement and increases in muscle enzymes.

> *Key Words: insulin* • *insulin sensitivity* • *endurance exercise* • *fat feeding*



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LIPOIC ACID AS AN ANTIOXIDANT IN MATURE THOROUGHBRED GELDINGS: A PRELIMINARY STUDY

C.A. WILLIAMS, R.M. HOFFMAN, D. S. KRONFELD, T.M. HESS, K.E. SAKER and P.A. HARRIS

 α -Lipoic acid (LA) has demonstrated antioxidant effects in humans and laboratory animals. The objective of this study was to determine whether the effects of LA are similar in horses. Five Thoroughbred geldings were supplemented with 10 mg/kg/d DL-a-lipoic acid in a molasses and sweet feed carrier and five received only the carrier as a placebo (CON). Blood samples were obtained at baseline (0 d), after 7 and 14 d of supplementation, and 48 h post-supplementation (16 d). Blood fractions of red and white blood cells (RBC and WBC, respectively) and plasma were analyzed for glutathione (GSH), glutathione peroxidase (GPx) and total plasma lipid hydroperoxides (LPO). An experienced veterinarian observed no adverse clinical effects. Plasma LPO baselines differed between groups (P=0.002). When covariates were used, there was a decrease over time in the LA group (P=0.015) and concentrations were lower in the LA group than in the CON group at 7 and 14 d (P=0.022 and P=0.0002, respectively). At baseline, GSH concentration was 69 ± 7 in WBC and 115 ± 13 mmol/mg protein in the RBC, with no differences resulting from either time or treatment. The GPx activity was 47 ± 4 and $26 \pm$ 5 U/g protein at baseline WBC and RBC, respectively, with a lower concentration in the LA group's WBC at 7 (P=0.019) and 14 d (P=0.013). The results show that 10 mg/kg LA had no evident adverse effects, and moderately reduced the oxidative stress of horses allowed light activity. These findings encourage studying of LA in horses subjected to strenuous exercise.

> Key Words: oxidative stress • horse • lipid hydroperoxides • glutathione • dihydrolipoate



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EFFECT OF DIETARY SUPPLEMENTS COMMONLY USED IN STANDARDBRED RACING ON PLASMA TOTAL CARBON DIOXIDE

V. BALASKONIS, A. SZUCSIK, R.A. LEHNHARD and K.H. McKEEVER

Ten mature Standardbred mares (9-13 years, ~522 kg) were used to test the hypothesis that pelleted dietary supplementation would alter total plasma bicarbonate (tCO2) concentrations. All the mares used in this study were unconditioned, but were familiarized to the laboratory setting and running on a treadmill. Each of the ten mares was semi-randomly assigned one of four dietary treatments. The four treatments (oats as control and three pelleted feed supplements: Drive, Omolene and Strategy) were administered in a crossover fashion throughout a 4-week testing period. These products were chosen based on the frequency of their use by Standardbred/Thoroughbred owners and trainers in New Jersey. The horses underwent a simulated race test (SRT) on a treadmill (6% grade) at the end of each administration period. During the SRT, horses ran for 2 min at 4 m/s, 2 min at the speed previously shown to correspond to VO_{2max} and 2 min at 4 m/s. Blood was collected before supplement treatment (24 h), 10 min prior to exercise and at 0, 60 and 90 min post-exercise. Plasma concentrations of bicarbonate, sodium, potassium and chloride were measured using a Beckman ELISE analyzer. The major finding of this study indicates that the plasma [tCO2], chloride and sodium concentrations were not altered by the dietary supplements studied (P>0.05). There were differences (P<0.05) in plasma [tCO2] across sampling intervals (-4h, -10 min, +0 min, +60 min and +90 min) that were attributable to acute exercise (mean \pm SE: 34.4 \pm 0.9, 33.2 ± 1.1 , 20.2 ± 0.8 , 31.5 ± 0.8 , 30.3 ± 1.6 mmol l21). There was a slight effect of treatment (P<-0.05) on potassium levels. However, exercise was the main factor that caused substantial changes (P<0.05) in the plasma tCO2, potassium, haematocrit and total protein concentrations. It was concluded that the pelleted diet supplements examined do not alter plasma [tCO2] in horses.

> *Key Words: equine* • *exercise* • *plasma total carbon dioxide* • *feed supplements*



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EFFECT OF SEVEN COMMON SUPPLEMENTS ON PLASMA ELECTROLYTE AND TOTAL CARBON DIOXIDE CONCENTRATION AND STRONG ION DIFFERENCE IN STANDARDBRED HORSES SUBJECTED TO A SIMULATED RACE TEST

A. SZUCSIK, V. BALISKONIS and K.H. McKEEVER

This study used a randomized crossover design, with investigators blind to the treatment given, to test the hypothesis that seven commercially available electrolyte supplements would alter plasma concentrations of Na⁺, K⁺, Cl⁻, lactate, total protein (TP) and total carbon dioxide (tCO2) as well as plasma strong ion difference (SID) and haematocrit (HCT). Ten unfit Standardbred mares (~450 kg, 4-9 years) completed a series of simulated race exercise tests (SRT) during which venous blood was collected at five sampling intervals (prior to receiving electrolyte treatment, prior to the SRT, immediately following exercise and at 60 and 90 min post-SRT). Plasma electrolyte and tCO2 concentrations were measured in duplicate using a Beckman EL-ISE electrolyte analyzer. No difference (P>0.05) between treatments was detected at any of the five sampling intervals for plasma [Na⁺], [K⁺], [Cl⁻] or [tCO2]. Similarly, no significant difference was detected between treatments across each of the five sampling intervals for plasma SID, HCT or TP concentration. There were differences (P<0.05) in plasma [Na^+], [K^+], and [tCO2] (as well as plasma SID, HCT, and TP concentration) in the immediately post-SRT samples that were attributable to the physiological pressures associated with acute exercise. No differences (P>0.05) were detected between treatments across the preelectrolyte and pre-SRT sampling intervals for plasma lactate concentration. There was, however, a significant time by treatment interaction during the 0, 60, and 90 min post-SRT sampling intervals for this parameter. The electrolyte supplements featured in this investigation did not affect either plasma tCO2 concentration or SID; however, this result does not rule out the potential for other supplements, especially those containing alkalinizing ingredients, to exert an effect that could push a horse towards threshold values.

> *Key Words: electrolyte* • *exercise* • *supplements* • *tCO2* • *strong ion difference*



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EFFECTS OF ORAL POTASSIUM SUPPLEMENTATION ON ACID-BASE STATUS AND PLASMA ION CONCENTRATIONS OF HORSES DURING ENDURANCE EXERCISE

T.M. HESS, D.S. KRONFELD, C.A. WILLIAMS, J.N. WALDRON, P.M. GRAHAM-THIERS, K. GREIWE-CRANDELL, M.A. LOPES and P.A. HARRIS

Objective: To compare effects of oral supplementation with an experimental potassiumfree sodium abundant electrolyte mixture (EM-K) with that of oral supplementation with commercial potassium-rich mixtures (EM+K) on acid-base status and plasma ion concentrations in horses during an 80-km endurance ride.

Animals: 46 healthy horses.

Procedure: Blood samples were collected before the ride; at 21-, 37-, 56-, and 80-km inspection points; and during recovery (i.e., 30-minute period after the ride). Consumed electrolytes were recorded. Blood was analyzed for pH, PvCO₂, and Hct, and plasma was analyzed for Na⁺, K⁺, Cl⁻, Ca2⁺, Mg2⁺, lactate, albumin, phosphate, and total protein concentrations. Plasma concentrations of H⁺ and HCO3⁻, the strong ion difference (SID), and osmolarity were calculated.

Results: 34 (17 EM-K and 17 EM+K treated) horses finished the ride. Potassium intake was 33 g less and Na+ intake was 36 g greater for EM-K-treated horses, compared with EM+K-treated horses. With increasing distance, plasma osmolarity; H⁺, Na⁺, K⁺, Mg2⁺, phosphate, lactate, total protein, and albumin concentrations; and PvCO₂ and Hct were increased in all horses. Plasma HCO3⁻, Ca²⁺, and Cl⁻ concentrations were decreased. Plasma H⁺ concentration was significantly lower in EM-K-treated horses, compared with EM+K-treated horses. Plasma K⁺ concentrations at the 80-km inspection point and during recovery were significantly less in EM-K-treated horses, compared with EM+Ktreated horses.

Conclusions and Clinical Relevance: Increases in plasma H+ and K+ concentrations in this endurance ride were moderate and unlikely to contribute to signs of muscle fatigue and hyperexcitability in horses.



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FEEDING MANAGEMENT PRACTICES AND SUPPLEMENT USE IN TOP-LEVEL EVENT HORSES

A.O. BURK and C.A. WILLIAMS

The objective of this study was to characterize the nutrition and feeding management practices, including concentrate and supplement use, of three-day event horses prior to and during high-level competition. Subjects were horses competing in one of two divisions at the Jersey Fresh Three-day Event in Allentown, NJ, in 2006 and/or 2007. Body weight and condition score of horses, and feeding management surveys, were completed prior to the start of competition with weight of hay and concentrate taken in 2007. Two sample t-tests were used to compare the data between divisions within each year of the study. For both years, the majority of horses were of the male gender and Thoroughbred breed, aged 11.1 ± 0.3 years. There were no differences between divisions within the year for any of the variables measured. The average numbers of supplements fed on a regular basis to horses competing in the 2006 Concours Complet d'Equitation International (CCI^{**}), 2006 CCI^{***}, 2007 CCI^{**} and 2007 CCI^{***} events were 4.2 ± 0.4 , 4.2 ± 0.3 , 4.2 ± 0.5 and 4.3 ± 0.7 , respectively. In both years, the most administered type of oral supplement was electrolytes, followed by plain salt and oral joint compounds. The majority of horses in both years did not have any change in their forage and concentrate feeding prior to being transported to the show facility. Feeding practices before and after cross-country varied between years and divisions, but most horses had feed withheld for about 2–4 h prior to the event. The majority of feeding management practices followed research-driven recommendations. However, the relatively high average supplement use per horse raises questions about over-supplementation and/or nutrient interactions in these horses.

> Key Words: equine • nutritional survey • supplements • three-day eventing • feeding management



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MICROHYDRIN SUPPLEMENTATION REDUCED PLASMA LACTATE IN EXERCISING STANDARDBRED MARES

C.A. WILLIAMS, E.D. LAMPRECHT, H. ROESSNER and K. LLOYD

Microhydrin[°] is a unique dietary supplement composed of silica, potassium, magnesium and vitamin C. Colloidal silicate minerals display a variety of properties which include an increase in surface area, bonding absorbed water as well as mineral ions. Structured water at the silica interface stabilizes electron transfer and creates a negative (-350 to -650mV) reduction/oxidation (redox) potentials. Specific silicate interactions play a substantial role in nutrient bioavailability by enhancing solubility properties, ion and water transport and provide free radical antioxidant protection. Microhydrin [°] was shown to reduce post exercise lactic acid concentrations in a human cross-over trial as compared to placebo. Lactic acid accumulation in the blood during heavy exercise in humans was shown to increase muscular acidosis and may inhibit optimal performance. Post-exercise effects of lactic acid have been shown to create muscle soreness during, immediately and one to two days after exercise. The purpose of this study was to evaluate the effect of Microhydrin [°] on lactic acid response in horses undergoing a sub-maximal treadmill exercise test.



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NUTRIENT INTAKE DURING AN ELITE LEVEL THREE-DAY EVENT COMPETITION IS CORRELATED TO INFLAMMATORY MARKERS AND ANTIOXIDANT STATUS

C. A. WILLIAMS and A. O. BURK

Reasons for performing study: Dietary intake and feeding management practices could affect the degree of physiological stress and subsequent performance of high-level 3-day event horses.

Objectives: To assess whether a relationship exists between dietary intake levels of selected nutrients and the inflammatory and antioxidant status in horses competing in a high level 3-day event.

Materials and methods: Riders competing in a CCI2*/CCI3* 3-day event (n=35) answered a nutritional management survey conducted by the investigators at the competition. Actual and recommended intakes of vitamin E, potassium (K), calcium (Ca), phosphorus (P) and magnesium (Mg) were calculated using the manufacturer or NRC values. Blood samples, bodyweight and body condition score of horses were taken pre-competition, 30 min and 18–24 h after cross country, but before stadium jumping. Data were analyzed using a mixed model ANOVA with repeated measures and Pearson's product moment correlation.

Results: Estimated daily intakes of vitamin E, K, Ca, P and Mg for horses were higher than daily recommended levels (P<0.05). In response to competition, tumour necrosis factor-a (TNF- α ; P=0.0002), nitric oxide (NO; P=0.013) and b-carotene (BC; P<0.0001) decreased, creatine kinase (P<0.0001) and aspartate aminotransferase (P=0.001) increased, and α -tocopherol and retinol did not change. Intake of vitamin E, K, Ca, P and bodyweight were negatively correlated with TNF- α (P<0.05). Vitamin E and bodyweight were also negatively correlated with NO (P<0.05). Pasture intake and BC were positively correlated (P<0.0001).

Conclusions: The decline in systemic inflammatory markers is probably due to increased utilization or excretion and decreased production related to the increased oxidative stress experienced by horses during competition. High bodyweights could also predispose horses to a higher level of inflammation during 3-day event competition.

Key Words:

 $horse \bullet antioxidant \bullet diet \bullet eventing \bullet inflammation \bullet nitric oxide \bullet nutrient intake$

tumour necrosis factor-α



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NUTRITION-ASSOCIATED PROBLEMS FACING ELITE LEVEL THREE-DAY EVENTING HORSES

E.R. LEAHY, A.O. BURK, E.A. GREENE and C.A. WILLIAMS

Reasons for performing study: The main goal of feeding elite 3-day event horses is to deliver nutrients in optimal amounts to allow the horse to maximize its health and performance. However, improper nutritional management and/or physiological stressors related to intense training and competition may increase the risk of nutrition-associated disorders in these horses. An understanding of the nutrition-associated problems contributing to poor performance is critical to the health and welfare of the horse.

Objectives: To characterize the nutrition-associated problems affecting top-level 3-day event horses during 2008.

Methods: Contact information for riders competing in the 2 highest levels of 3-day eventing in 2008 was obtained from the United States Eventing Association. A survey containing 10 questions pertaining to participant demographics and nutrition-associated problems experienced by their horses was mailed and e-mailed to the 81 individuals fitting our criteria of living in USA and Canada. Data was collected in April and May 2009.

Results: Twenty-nine of 81 riders completed the survey (35.8%). Respondents rode a total of 45 horses in top level 3-day events in 2008. The top 5 nutrition-associated problems that horses faced at a significantly higher level than the other problems (P<0.0001) were gastric ulcers (42.2%), joint problems (37.7%), decreased appetite (31.1%), weight loss (31.1%) and hyperexcitability (22.2%). There was no significant difference in frequency of problems between home and competition (P=0.22).

Conclusions: Horses competing at a high level of 3-day eventing in 2008 were at risk of reduced performance given the significant rate of gastric ulcers, decreased appetite and weight loss. Research addressing specific causes of and/or feeding management changes that would reduce the incidence of these problems in these horses is needed to ensure optimal health and performance.

Key Words: horse • *appetite* • *eventing* • *nutrition* • *ulcers* • *survey*



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POTASSIUM-FREE ELECTROLYTES AND CALCIUM SUPPLEMENTATION IN AN ENDURANCE RACE

T.M. HESS, K.M. GREIWE-CRANDELL, J.E. WALDRON, C.A. WILLIAMS, M.A. LOPES, L.S. GAY, P.A. HARRIS and D.S. KRONFELD

Some of the clinical signs seen in horses during endurance races may result from increases in neuromuscular excitability and are related to plasma $[K^+]$ and $[Ca^{++}]$. The present study aimed to test the following hypotheses: (1) Potassium supplementation will affect plasma $[K^+]$ and may result in clinical signs related to neuromuscular hyperexcitability during an 80 km endurance ride. (2) Plasma [Ca⁺⁺] will reflect dietary cation–anion balance (DCAB) and calcium intake. Feeding with a high DCAB and high dietary calcium content (1.5% total calcium of daily ration) diets would lead to higher plasma [Ca⁺⁺] during an endurance race than on feeding high DCAB diets with a moderate dietary calcium content (1% of total calcium of daily ration). The current study was undertaken during the 80 km endurance research ride in 2002 in Virginia, USA. Forty volunteer rider-horse pairs participated in the race. During the race, electrolyte mixtures with (EM + K) and without (EM - K) potassium were supplied to 18 and 22 horses, respectively. After the race, the horses receiving EM - K during the race were supplied with a recovery formula containing potassium (EM-REC). The horses were fed in addition to their own forage (hay and pasture) either their own commercial concentrate (CC; 1% calcium, n ¹/₄ 11) or one of two research-supplied concentrates during 3 months preceding the research ride, one concentrate rich in sugar and starch (SS; 2% calcium, n ¼ 15) and the other rich in fat and fibre (FF; 2% calcium, n=14). Peripheral blood samples were taken the day before, within 3 min of the arrival at the vet checks at 27, 48 and 80 km, and after 3 h of recovery. Plasma samples were analyzed for pH, haematocrit (Hct), [Na⁺], [K⁺], [Cl⁻], [Ca⁺⁺], [Mg⁺⁺], total protein (TP) and albumin [alb]. Effects of sampling times, treatments and interactions were evaluated by ANOVA in a mixed model with repeated measures and applied to the 25 horses that completed 80 km. Eliminated horses had their blood sampled before entering the elimination vet check and 3 h after elimination, and were compared with finishing horses by t-test. As the ride progressed, significant increases were found in plasma pH, [Na⁺], [PO⁻₄], [TP], [alb], Hct and osmolality; and decreases in [K⁺], [Mg⁺⁺], PCO₂, [Ca⁺⁺] and [Cl⁻]. Horses supplied with potassium-free, sodium-rich electrolyte formulae (EM - K) had 12.5% lower (P=0.001) mean plasma [K⁺], 7.8% lower (P=0.024) TP and 8.4% lower (P=0.004) albumin at 80 km, and at 3 h after the race they had 6.8% lower (P=0.045) TP, when compared with EM + K supplemented horses. Horses fed with SS and FF had higher $[Ca^{++}]$ at 27 (P=0.027), 56 (P=0.006) and 80km (P=0.022) when compared with horses fed with CC. The lower [K⁺] in the EM - K group, and the higher [Ca⁺⁺] in the SS- and FF-supplemented horses may help prevent increases in neuromuscular excitability and related clinical signs. The lower TP and albumin indicate less dehydration in the EM - K group and could help prevent related disorders.

Key Words:

horse • $[Ca^{**}] \bullet [K^*] \bullet DCAB \bullet$ *neuromuscular excitability* • *electrolytes*



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SUPEROXIDE DISMUTASE SUPPLEMENTATION DOES NOT AFFECT ERYTHROCYTE SUPEROXIDE DISMUTASE ACTIVITY OR SYSTEMIC INFLAMMATORY CYTOKINE RESPONSES IN AN EQUINE EXERCISE MODEL

E.D. LAMPRECHT, C.A. BAGNELL and C.A. WILLIAMS

Both exercise and inflammatory processes have been shown to induce oxidative stress, which if left unresolved can result in chronic degenerative disorders.¹ Superoxide dismutase (SOD) is an antioxidant enzyme that catalyzes the dismutation of superoxide anions into oxygen and hydrogen peroxide, preventing the formation of hydroxyl and peroxynitrite radicals. Supplementation of SOD in other species has been shown to be beneficial in reducing oxidative stress and inflammation, however data from equine trials are needed. Objectives of this study were to evaluate effects of exercise and oral SOD supplementation on erythrocyte SOD activity and inflammatory cytokine responses in horses.

Key Words: equine • *oxidative stress* • *inflammation* • *exercise*



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CHANGES IN CONCENTRATIONS OF NEUROENDOCRINE HORMONES AND CATECHOLAMINES IN DOGS WITH MYOCARDIAL FAILURE INDUCED BY RAPID VENTRICULAR PACING

B.M. ROCHE, D. SCHWARTZ, R.A. LEHNHARD, K.H. McKEEVER, T. NAKAYAMA, T.E. KIRBY, P.L. ROBITAILLE and R.L. HAMLIN

Objective: To describe neuroendocrine responses that develop in dogs subjected to prolonged periods of ventricular pacing.

Animals: 14 adult male hound-type dogs.

Procedure: Samples were obtained and neuroendocrine responses measured before (baseline) and after 3 periods of ventricular pacing. A pacemaker was used to induce heart rates of 180, 200, and 220 beats/min (BPM). Each heart rate was maintained for 3 weeks before increasing to the next rate. Atrial natriuretic peptide, antidiuretic hormone, aldosterone, norepinephrine, epinephrine, and dopamine concentrations and plasma renin activity were measured. Severity of left ventricular compromise was estimated.

Results: Shortening fraction decreased significantly with increasing heart rates (mean \pm SE, 35.5 \pm 1.4, 25.0 \pm 1.4, 19.5 \pm 1.9, and 12.2 \pm 2.3 for baseline, 180 BPM, 200 BPM, and 220 BPM, respectively). Atrial natriuretic peptide concentrations increased significantly at 180 BPM (44.1 \pm 3.0 pg/mL) and 200 BPM (54.8 \pm 5.5 pg/mL), compared with baseline concentration (36.8 \pm 2.6 pg/mL). Dopamine concentration increased significantly at 200 BPM (70.4 \pm 10.4 pg/mL), compared with baseline concentration (44.2 \pm 7.3 pg/mL). Norepinephrine concentrations increased significantly from baseline concentration (451 \pm 46.2 pg/mL) to 678 \pm 69.8, 856 \pm 99.6, and 1,003 \pm 267.6 pg/mL at 180, 200, and 220 BPM, respectively.

Conclusions and Clinical Relevance: Dogs subjected to ventricular pacing for 9 weeks developed neuroendocrine responses similar to those that develop in humans with more chronic heart failure and, except for epinephrine concentrations, similar to those for dogs subjected to ventricular pacing for <6 weeks.



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THE EFFECT OF HEAD-DOWN TILT AND WATER IMMERSION ON INTRACRANIAL PRESSURE IN NONHUMAN PRIMATES

L.C. KEIL, K.H. MCKEEVER, M.G. SKIDMORE, J. HINES and W.B. SEVERS

A headward fluid shift occurs in humans exposed to spaceflight. This shift is thought to be the stimulus for the observed reduction in plasma volume, and may produce a sustained rise in intracranial pressure (ICP). In order to determine the effect of headdown tilt (HDT) and water immersion on intracranial pressure (ICP), five anesthetized rhesus monkeys were fitted with intracronial pressure-monitoring transducers and subjected to -6° HDT followed by head-out thermoneutral (34.7) water immersion. ICP was elevated from 3.8 ± 1.1 mm Hg in the horizontal control period to 5.3 ± 1.3 mm Hg (P<0.05) during the 15 rain pre-immersion HDT. When seated in the empty immersion tank, ICP stabilized at -6.3 \pm 1.3 mm Hg for the control period and increased to -2.2 \pm 1.9 mm Hg (P < 0.05) when the water level was maintained for 30 rain at the sternal notch. The ICP returned toward pre-immersion levels ($-5.5 \pm 1.4 \text{ mm Hg}$) as the tank was drained. Mean blood pressure (MBP) remained constant during the pre-immersion tilt test. In the pre-immersion control period, MBP was $91 \pm 3 \text{ mm}$ Hg compared to 90 ± 4 mm Hg (P>0.05) during immersion and 82 ± 1 mm Hg (P<0.05) in the post-immersion recovery period. In summary, exposure of rhesus monkeys to either head-down tilt or water immersion produced a prompt rise in ICP during the stimulus period. The magnitude of the increased ICP was greater with water immersion than with -6° HDT. However, ICP did not appear to rise to pathological levels.



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EXERCISE TRAINING-INDUCED HYPERVOLEMIA IN GREYHOUNDS: ROLE OF WATER INTAKE AND RENAL MECHANISMS

K. H. McKEEVER, W.A. SCHURG and V.A. CONVERTINO

The purpose of this study was to determine if the chronic hypervolemia that accompanies endurance exercise training is due only to an increase in the rate of water intake or if there were contributions from renal mechanisms. Four greyhound dogs, previously sedentary for 3 yr, were utilized. During the 28-day experiment each dog was trained on a treadmill ergometer for 14 consecutive days at 65% of its pre-training maximal work intensity. After training, plasma volume increased 472 ml (27.5%, P less than 0.05). The rate of water intake increased 328 ml/day (33%, P<0.05), whereas urine output increased 87 ml/day (20.8%, P<0.05). The mean resting 24-h values for clearance of sodium increased 0.29 ml/min (90.3%, P<0.05), and clearance of potassium decreased 1.51 ml/min (16.1%, NS). Glomerular filtration rate, free water clearance, and osmotic clearance were not significantly altered. These data suggest that the primary mechanism for the exercise training-induced hypervolemia in dogs is a net positive water balance via increased water consumption without significant contribution from an increase in renal water reabsorption.

Key Words: dogs • *renal function*



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INTRAPERICARDIAL DENERVATION: RESPONSES TO WATER IMMERSION IN RHESUS MONKEYS

K.H. McKEEVER, L.C. KEIL and H. SANDLER

Eleven anesthetized rhesus monkeys were used to study cardiovascular, renal, and endocrine alterations associated with 120 min of head-out water immersion. Five animals underwent complete intrapericardial denervation using the Randall technique, while the remaining six monkeys served as intact controls. Each animal was chronically instrumented with an electromagnetic flow probe on the ascending aorta, a strain gauge pressure transducer implanted in the apex of the left ventricle (LV), and electrocardiogram leads anchored to the chest wall and LV. During immersion, LV enddiastolic pressure, urine flow, glomerular filtration rate, sodium excretion, and circulating atrial natriuretic peptide (ANP) each increased (P<0.05) for intact and denervated monkeys. There were no alterations in free water clearance in either group during immersion, yet fractional excretion of free water increased (P<0.05) in the intact monkeys. Plasma renin activity (PRA) decreased (P<0.05) during immersion in intact monkeys but not the denervated animals. Plasma vasopressin (PVP) concentration decreased (P<0.05) during the first 30 min of immersion in both groups but was not distinguishable from control by 60 min of immersion in denervated monkeys. These data demonstrate that complete cardiac denervation does not block the rise in plasma ANP or prevent the natriuresis associated with head-out water immersion. The suppression of PVP during the first minutes of immersion after complete cardiac denervation suggests that extracardiac sensing mechanisms associated with the induced fluid shifts may be responsible for the findings.

> Key Words: water immersion • natriuresis • vasopressin • cardiac denervation • monkey



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USING WEB 2.0 TECHNOLOGY (YOUTUBE) TO REACH EXTENSION CLIENTELE

E.A. GREENE, J.L. WHITTLE, A.S. GRIFFIN, C.H. WOOD, K. ANDERSON and C.A. WILLIAMS

YouTube[™] was created in 2005 as a video sharing website where users can upload, view and share video clips. It has become the clear leader in online video sharing with a collection of more than 100 million video clips with 65,000 videos added daily. YouTube[™] currently operates as a subsidiary of Google¹ and uses Adobe Flash video technology to display movie, video, TV and other video clips. Revenue is earned by selling advertising and partnership deals with content providers such as CBS, BBC, Universal Music Group, Sony Music Group, Warner Music Group, NBA, and The Sundance Channel.

> *Key Words: YouTube*[™] • *eXtension* • *video* • *viral marketing*



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CHANGES IN ADIPOPNECTIN, LEPTIN, AND FAT MASS AFTER Clenbuterol Treatment in Horses

C.F. KEARNS, K.H. McKEEVER and K. MALINOWSKI

Introduction: Adipose tissue plays complex role(s) in metabolic and endocrine control. To date, little work has been done in the horse regarding adipocytokines.

Purpose: This study was conducted to determine whether therapeutic levels of chronic β -agonist administration, exercise, or both could alter their concentrations.

Methods: A total of 23 standard-bred mares were divided into four experimental groups: clenbuterol (2.4 μ g•kg⁻¹ bw twice daily for 8 wk) plus exercise (8 wk, 20 min•d⁻¹ at 50% VO_{2max}; CLENEX; N=6), clenbuterol only (CLEN; N=6), exercise only (EX; N=5), and control (CON; N=6). Rump fat thickness was measured using B-mode ultrasound and percent body fat (%fat) was calculated. Plasma adiponectin and leptin concentrations were measured using radioimmunoassay (RIA). In the absence of purified equine adiponectin or leptin, results were expressed as human equivalents of immunoreactive adipocytokines.

Results: The change in plasma immunoreactive (ir)-adiponectin HE concentration was negatively correlated (r=-0.520; P=0.01) to the change in fat mass and positively correlated (r=0.446; P=0.03) to the change in fat-free mass. The change in plasma irleptin HE concentration was positively correlated (r=0.550; P=0.02) to the change in fat mass and negatively correlated (r=-0.473; P G 0.05) to the change in fat-free mass.

Conclusion: These data demonstrate that a chronic clenbuterol administration alters the concentrations of the adipocytokines adiponectin and leptin in horses. These changes may play a role in previously reported repartitioning effects of clenbuterol.

Key Words: equine • *adipocytokines* • *training*



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CHRONIC ADMINISTRATION OF THERAPEUTIC LEVELS OF CLENBUTEROL ACTS AS A REPARTITIONING AGENT

C.F. KEARNS, K.H. McKEEVER, K. MALINOWSKI, M.B. STRUCK and T. ABE

The purpose of this study was to examine the effect of therapeutic levels of clenbuterol, with and without exercise training, on body composition. Twenty-three unfit Standardbred mares were divided into four experimental groups: clenbuterol (2.4 µg/kg body wt twice daily) plus exercise (ClenEx; 20 min at 50% maximal oxygen consumption 3days/wk; n=6), clenbuterol only (Clen; n=6), exercise only (Ex; n=5), and control (Con; n=6). Rump fat thickness was measured at 2-wk intervals by using B-mode ultrasound, and percent body fat (% fat) was calculated by using previously published methods. For Ex, body fat decreased (P<0.05) at week 4 (-9.3%), % fat at week 6 (-6.9%), and fat-free mass (FFM) increased (P<0.05) at week 8 (+3.2%). On the other hand, Clen had significant changes in %fat (-15.4%), fat mass (-14.7%), and FFM (+4.3%) at week 2. ClenEx had signifi- cant decreases in %fat (-17.6%) and fat mass (-19.5%) at week 2, which was similar to Clen; however, this group had a different FFM response, which significantly increased (+4.4%) at week 6. Con showed no changes (P>0.05) in any variable at any time. These results suggest that exercise training and clenbuterol have additive effects with respect to %fat and fat mass but antagonistic effects in terms of FFM. Furthermore, chronic clenbuterol administration causes sig- nificant repartitioning in the horse, even when administered in therapeutic doses.

> *Key Words: ultrasound* • *equine* • β2-agonist



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CHRONIC CLENBUTEROL ADMINISTRATION ALTERS MYOSIN HEAVY CHAIN COMPOSITION IN STANDARDBRED MARES

M.D. BEEKLEY, J.M. IDEUS, W.F. BRECHUE, C.F. KEARNS and K.H. McKEEVER

The purpose of this study was to examine changes in myosin heavy chain (MHC) composition due to chronic clenbuterol administration with or without exercise in mares. Unfit Standardbred mares (aged 10 ± 3 years) were divided into four groups: clenbuterol (2.4 μ /kg BW twice daily) plus exercise (3 days/week for 20 min at 50% VO2_{max}; CLENEX; n=6), clenbuterol only (CLEN; n=6), exercise only (EX; n=5), and control (CON; n=6). Muscle biopsies were obtained from gluteus medius muscle before and after the eight-week training/administration period. MHC composition was determined via SDS gel electrophoresis and quantified using a scanning and densometric system.

CLENEX and CLEN exhibited significant (P<0.05) MHC changes while EX and CON did not. MHC type IIA decreased (29.8 \pm 6.1 to 19.3 \pm 4.0%, CLENEX; and 36.8 \pm 12.4 to 26.4 \pm 7.9%, CLEN) and MHC type IIX increased (59.4 \pm 7.2 to 71.8 \pm 5.8%, CLENEX; and 50.5 \pm 12.5 to 62.0 \pm 9.3%, CLEN). Chronic clenbuterol administration with and without exercise resulted in a significant shift in MHC profile in Standardbred mares.

> *Key Words: aerobic* • *horse* • *fiber type* • *performance* • *bronchodilator*



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CHRONIC CLENBUTEROL ADMINISTRATION NEGATIVELY ALTERS CARDIAC FUNCTION

M.M. SLEEPER, C.F. KEARNS and K.H. McKEEVER

Purpose: Chronic administration of pharmacological levels of β 2-agonists have been shown to have toxic effects on the heart; however, no data exist on cardiac function after chronic clenbuterol administration. The purpose of this study was to examine the effect of therapeutic levels of clenbuterol on cardiac performance.

Methods: Twenty unfit Standardbred mares were divided into four experimental groups: clenbuterol (2.4 μ g·kg⁻¹ twice daily 5 d·wk⁻¹) plus exercise (20 min at 50% VO_{2max}) (CLENEX; n=6), clenbuterol (CLEN; n=6), exercise (EX; n=4), and control (CON; n=4). M-mode and two-dimensional echocardiography (2.5-MHz sector scanner transducer) were used to measure cardiac size and function before and immediately after an incremental exercise test, before and after 8 wk of drug and/or exercise treatments.

Results: After treatment, CLENEX and CLEN demonstrated significantly higher left ventricular internal dimension (LVD) at end diastole ($+23.7 \pm 4.8\%$; $+25.6 \pm 4.1\%$), LVD at end systole ($+29.2 \pm 8.7\%$; $+40.1 \pm 7.9\%$), interventricular septal wall thickness (IVS) at end diastole ($+28.9 \pm 11.0\%$; $+30.7 \pm 7.0\%$), IVS at end systole ($+29.2 \pm 8.7\%$; $+40.1 \pm 7.9\%$), and left ventricular posterior wall systolic thickness ($+43.1 \pm 14.\%$; $+45.8 \pm 14.1\%$). CLENEX and CLEN had significantly increased aortic root dimensions ($+29.9 \pm 6.1\%$; $+24.0 \pm 1.7\%$), suggesting increased risk of aortic rupture.

Conclusion: Taken together, these data indicate that chronic clenbuterol administration may negatively alter cardiac function.

Key Words: echocardiography • *exercise testing* • *equine* • *clenbuterol*



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CHRONIC RECOMBINANT EQUINE SOMATOTROPIN (EST) Administration does not affect aerobic capacity or exercise performance in geriatric mares

K.H. McKEEVER, K. MALINOWSKI, R.A. CHRISTENSEN and H.D. HAFS

The purpose of this experiment was to test the hypothesis that chronic (89 days) administration of recombinant equine somatotropin (eST) would increase aerobic capacity and improve exercise performance in old snares. Fifteen, healthy, unfit, aged (20–26 year old) mares were randomly assigned to a treatment (eST, 12.5 mg day⁻¹ in 3 ml glycine/manitol buffer, SC, n=7) or control (vehicle, 3 ml day⁻¹, SC, n=8) group. Aerobic capacity and exercise performance were measured using a standardized exercise test (SET) performed on a high speed treadmill. Tests were conducted before (-21 days), during (+43 days and +89 days) and after (+127 clays) treatment. During the SET, resting data were collected and the horses then ran up a fixed 6% grade, starting at 4 m s⁻¹, with a 1 m s⁻¹ increase every 60 s (omitting 5 m s⁻¹) until fatigue. Oxygen uptake (VO₂) was measured using an open flow calorimeter and blood lactate concentration (LA) via a lactate analyser. Venous blood samples (10 ml) were collected at rest, during the last 10 s of each step of the SET, and after exercise and used to measure LA, plasma protein concentration (PP), hematocrit (HCT), and the plasma concentrations of creatine kinase (CK) and aspartamine transferase (AST). There were no differences (P>0.05) in resting VO₂, LA, TPP, or HCT due to treatment or test time. Furthermore, there were no differences (P>0.05) in maximal oxygen uptake (VO_{2max}), top run velocity, run time, watts at VO_{2max} velocity to produce a lactate of 4 mmoll⁻¹ (VLA4), watts at VLA4, peak HCT or peak LA. Finally, there were no differences (P>0.05) in resting or post-exercise CK or AST. These data indicate that chronic eST administration does not affect aerobic capacity or indices of exercise performance in unfit aged mares.

Key Words:

horse • *somatotropin* • *aerobic capacity* • *exercise performance*



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CLENBUTEROL AND THE HORSE: REVISITED

C.F. KEARNS and K.H. MCKEEVER

Clenbuterol is a β_2 -agonist and potent selective bronchodilator that is used to treat bronchospasm in the horse. The drug is normally administered to horses orally as a syrup formulation. Once absorbed into the systemic circulation, clenbuterol has the potential to cause many side effects, including a repartitioning effect and major alterations in cardiac and skeletal muscle function. Recent studies have also reported that clenbuterol can affect bone and the immune, endocrine and reproductive systems. A great deal of information has been published on the beneficial effects of short term therapeutic doses of clenbuterol on the equine respiratory system, although there is limited information about chronic administration, particularly since this has been associated with adverse physiological effects on other systems. This review summarizes the relevant understanding of clenbuterol for clinicians and horse owners who may administer this drug to pleasure and performance horses.

Key Words: clenbuterol • β_2 -agonist • horse • equine



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CLENBUTEROL DIMINISHES AEROBIC PERFORMANCE IN HORSES

C.F. KEARNS and K.H. McKEEVER

Purpose: The purpose of this 8-wk study was to examine the effect of therapeutic levels of clenbuterol on aerobic performance and hemodynamics associated with exercise.

Methods: Twenty-three unfit Standardbred mares were divided into four experimental groups, clenbuterol ($2.4 \ \mu g \cdot kg^{-1}$ body weight twice daily) plus exercise (20 min at 50% VO_{2max}; CLENEX; n=6), clenbuterol only (CLEN; n=6), exercise only (EX; n=5), and control (CON; n=6). All horses performed an incremental exercise test (GXT) to measure maximal oxygen consumption (VO_{2max}), blood lactate concentration, total plasma protein concentration, and hematocrit. Plasma volume, heart rate, right ventricular pressure (RVP), and pulmonary artery pressure (PAP) were measured before and after the treatment/training. Each horse also performed an exercise capacity test (ECT) in which they ran at their pretreatment VO_{2max} speed until exhausted.

Results: There were no significant changes in blood lactate, total protein, or hematocrit for any group during either the GXT or ECT. CLENEX decreased (P<0.05) VO_{2max} (-6.2%) and velocity to VO_{2max} (-10.0%), whereas both CLENEX and CLEN decreased (P<0.05) in time to exhaustion (-20.5 ± 4.7 and -20.9 ± 5.6%). EX alone increased (P<0.05) VO_{2max} (+6.5%), velocity to VO_{2max} (+10.0%), velocity to produces lactate concentration of 4 mmol (+13.5%), and time to exhaustion (+32.3 ± 15.0%). Plasma volume was altered (P<0.05) in CLENEX (-10%) and EX (+27%) but not in CLEN. Posttest recovery HR was higher (P<0.05) at 2 min post-GXT in the CLENEX, CLEN, and CON compared with their pretest values; RVP remained elevated at 2 min of recovery in the CLEN and CON groups; however, in the EX, recovery HR and RVP had returned to pre-GXT levels by 2 min of recovery.

Conclusions: These data suggest that the combined effect of therapeutic levels of clenbuterol and training decrease aerobic performance and that the resultant reduction in plasma volume may affect improvements in cardiovascular function during recovery normally seen with exercise training.

Key Words: equine • *exercise testing* • *hemodynamics* • β*-agonist*



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CROSS-REACTIVITY BETWEEN HUMAN ERYTHROPOIETIN ANTIBODY AND HORSE ERYTHROPOIETIN

C.F. KEARNS, J.A. LENHART and K.H. McKEEVER

Erythropoietin (EPO) is the primary hormone of erythropoiesis. Administration of recombinant human erythropoietin (rhuEPO) to improve racing performance in the horse represents a new form of blood doping, which has been associated with increased mortality. While immunoassay kits have become plentiful, very few commercial hormone assays are made specifically for equine research. There is a strong degree of sequence homology reported for EPO among species, which has allowed antibodies designed for human EPO research to be used to determine EPO concentration in other species. The objective of the present study was to use Western blot analysis to determine whether the antibody to rhuEPO, provided in a commercial radioimmunoassay (RIA) kit, recognizes horse EPO. Western blot analysis of purified rhuEPO and horse plasma was conducted, using the polyclonal goat-antihuman EPO antibody supplied in the Incstar EPO Trac RIA as the primary antibody. Immunoblot analysis revealed a major band at approximately 52 kDa for both rhuEPO and the horse plasma. Our results demonstrate that a human EPO antibody recognizes equine EPO. These findings show that the Incstar EPO Trac RIA hormone assay system can be used to measure equine EPO.

Key Words: erythropoietin • *western blot* • *radioimmunoassay*



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Detection of pegylated epoetin $\boldsymbol{\beta}$ in horse plasma after intravenous administration

D.H. CATLIN, G.A. MAYLIN, S. BENCHAAR, S.M. NEADES, M.S. TIMMONS and K.H. MCKEEVER

Methoxy polyethylene glycol–epoetin β (pegylated epoetin β , peg-epoetin β , Mircera) is an erythropoiesis-stimulating agent that is synthesized by pegylating epoetin β . It is prohibited in racehorses and humans because it enhances their performance. It is detectable in human urine and plasma using the isoelectric focusing–double-blotting (IEF–DB) method that has been widely applied in human doping control laboratories to detect doping with recombinant erythropoietins (EPOs). Fifty micrograms of peg-epoetin β were administered intravenously to a 450 kg female Thoroughbred, and blood was collected for 48 h. The plasma was processed by immunopurification followed by IEF– DB. The analytical procedures required about 2 days. No peg-epoetin β was detected in the baseline plasma collected prior to drug administration. It was readily detected in samples collected at 2, 4, 6, 8, 24 and 48 h. The isoforms showed a characteristic pattern that differed from epoetin β and was unlike any other known erythrocyte-stimulating agent or recombinant EPO. No peg-epoetin β was detected in plasma from five control horses. The plasma samples were also tested by a rapid, automated and chemiluminescent immunometric assay for EPO (Immulite). The samples collected from the treated horse at

2–48 h contained the equivalent of approximately 200–800 pg ml⁻¹ of peg-epoetin β . The samples from the ten control horses were negative. These data show that a small dose of peg-epoetin β is detectable for 48 h by two different methods that are based on two different principles (chemiluminescence immunoassay and IEF–DB). The Immulite assay is suitable as a screen and the IEF–DB assay as a confirmation method. These studies need to be expanded to other horses. The screen and confirmation tests, if applied, will probably eliminate the use of peg-epoetin β as a horse doping agent.

Key Words: horse • doping • erythropoietin • recombinant polyethylene glycol–epoetin β



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EFFECT OF CHRONIC CLENBUTEROL ADMINISTRATION AND EXERCISE TRAINING ON IMMUNE FUNCTION IN HORSES

K. MALINOWSKI, C.F. KEARNS, P.D. GUIRNALDA, V. ROEGNER and K.H. McKEEVER

Effects of longitudinal exercise training and acute intensive exercise (simulated race test) on immune function have not been reported in horses. Clenbuterol, a β_2 -adrenergic agonist, is used to manage inflammatory airway disease in horses. This study investigated the interaction of 8 wk of exercise training with or without 12 wk of clenbuterol administration in horses. Twenty-three untrained standardbred mares (10 ± 3 yr, mean \pm SE) were used and divided into four experimental groups. Horses given clenbuterol plus ex- ercise (CLENEX; n=6) and clenbuterol alone (CLEN; n=6) received 2.4 µg/kg BW of clenbuterol twice daily (in an average volume of 20 mL) on a schedule of 5 d on and 2 d off for 12 wk. The CLENEX group was also aerobically trained 3 d/wk. Mares given exercise alone (EX; n=5) were aerobically trained for 3 d/wk, and the control group (CON; n=6) remained sedentary. Both EX and CON horses were administered similar volumes (approximately 20 mL) of molasses twice daily. A simulated race test (SRT) resulted in an elevation in lymphocyte number post-exercise (P<0.05). There was no significant difference after acute exercise in either monocyte or granulocyte number. Acute exercise resulted in a decrease (P<0.05) in the percentage of CD4+ and an increase (P<0.05) in the percentage of CD8+ cells. The SRT resulted in a decreased lymphoproliferative response to pokeweed mitogen (P<0.05). A SRT had no effect on antibody production in response to equine influenza vaccine. The EX group demonstrated greater cortisol concentrations at rest and at all other time points postexercise after completing the training regimen compared with CLENEX horses (P<0.05). Pre-exercise (SRT) peripheral blood monocyte number was lower in CLENEX horses than in other treatment groups (P<0.05). Clenbuterol and exercise training did not significantly affect post-SRT changes in leukocyte numbers. Exercise training resulted in a decrease (P<0.05) in the percentage of CD8+ cells post-SRT com- pared with other groups, but the percentage of CD4+ cells was not altered by either clenbuterol or exercise conditioning. Lymphocyte proliferative response was not affected by clenbuterol or exercise treatment. Horses demonstrated responses to bouts of acute exercise as noted with other species, namely humans and rodents.

> *Key Words: adrenergic agonist* • *equine* • *exercise* • *exertion* • *stress*



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EFFECT OF FUROSEMIDE AND WEIGHT CARRIAGE ON ENERGETIC RESPONSES OF HORSES TO INCREMENTAL EXERTION

K.W. HINCHCLIFF, K.H. McKEEVER, W.W. MUIR III and R. SAMS

The effect of furosemide-induced weight loss on the energetic responses of horses to running was examined in a 3-way crossover study. Eight 2- to 3-year-old Standardbred mares received, in random order, 10 ml of saline solution 4 hours before running on a treadmill (control trial, C); or, during 2 trials, 1 mg of furosemide/kg of body weight, i.v., 4 hours before running. During one of the trials when the horses received furosemide, they carried weight equal to that lost over the 3.75 hours after furosemide administration while running (furosemide-loaded, FL), and during the other trial they did not carry weight equal to that lost after furosemide administration (furosemide-unloaded, FU). Horses performed an incremental exercise test on a treadmill during which rates of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured, respiratory exchange ratio was calculated, and blood samples were collected for determination of mixed venous plasma lactate concentration and arterial and mixed venous oxygen saturation. Furosemide treatment caused significantly (P<0.001) greater weight loss than did saline administration; mean ± SEM weight loss (exclusive of fecal loss) was 1.6, 8.8, and 10.2 kg (SEM=2.0) for C, FL, and FU trials, respectively. The speed at which peak VO₂ was achieved was 9.31, 9.56, and 9.50 (SEM=0.16) m/s, respectively, time to fatigue was 547, 544, and 553 (SEM=26) seconds, respectively, and the highest speed attained was 10.3, 10.2, and 10.2 (SEM=0.2) m/s, respectively. Mean peak rate of oxygen consumption was 130.7, 129.6, and 129.6 (SEM=1.9) ml/min/kg, respectively.



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EFFECT OF FUROSEMIDE ON PLASMA ATRIAL NATRIURETIC PEPTIDE AND ALDOSTERONE CONCENTRATIONS AND RENIN ACTIVITY IN RUNNING HORSES

J.L. COOLEY, K.W. HINCHCLIFF, K.H. McKEEVER, D.R. LAMB, and W.W. MUIR III

Effects of furosemide administration on exertion-induced changes in plasma renin activity and plasma concentrations of atrial natriuretic peptide and aldosterone in horses during sustained submaximal exertion were examined. Furosemide (1 mg/kg of body weight) or heparinized saline solution was administered IV to each of 6 mares not conditioned to exercise, either 4 hours or 2 minutes before 60 minutes of sustained submaximal running on a treadmill. Horses ran at a speed that induced heart rate approximately 65% of maximal after saline treatment. After 15 minutes of running, furosemide suppressed the exertion-induced increase in plasma concentrations of atrial natriuretic peptide (mean [95% confidence interval] values of 63.9 [9.9 to 421] pg/ml vs 100 [15.4 to 652] pg/ml after furosemide or saline treatment, respectively), and enhanced the response of plasma renin activity to exertion (18.6 [5.7 to 60.4] ng/ml/h vs 6.0 [1.8 to 19.4] ng/ml/h, respectively). An effect of furosemide on the exertion-induced increase in plasma aldosterone concentration was not detected.



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EFFECT OF OMEPRAZOLE AND RANITIDINE ON TOTAL CARBON DIOXIDE CONCENTRATION IN HORSES SUBJECTED TO A SIMULATED RACE TEST

D. CIOLINO, R.A. LEHNHA and K.H. McKEEVER

The purpose of this study was to test the hypothesis that the gastric ulcer medications, ranitidine and omeprazole, would alter plasma concentrations of total carbon dioxide

(tCO₂), lactate (LA), Na⁺, K⁺, Cl⁻ and total protein (TP), as well as calculated plasma strong ion difference (SID) and packed cell volume (PCV) in horses subjected to a simulated race test (SRT). Twelve unfit Standardbred mares (~520 kg, 9–18 years) were used in a randomized crossover design with the investigators blind to the treatment given. Each mare received a treatment three times daily (TID) at 06.30, 12.30 and 18.30 hours. The treatments administered orally were omeprazole (4 mg/kg was given in the morning with apple sauce given at the later, two dosing times to encourage good behavior), ranitidine (6 mg/kg crushed and mixed in 20 ml apple sauce) and control (20 ml apple sauce TID). Each horse completed a series of SRTs with blood samples taken via jugular venipuncture at five intervals (prior to receiving treatment, prior to SRT, immediately following exercise and at 60 and 90 min post-SRT). During the SRTs, each horse ran on a treadmill fixed on a 6% grade for 2 min at a warm-up speed (4 m/s) and then for 2 min at a velocity predetermined to produce VO_{2max}. Each horse then walked at 4 m/s for 2 min to complete the SRT. Plasma tCO₂, electrolytes, LA and TP concentrations and PCV-TP were measured in duplicate at all intervals. No differences (P>0.05) were detected between control, ranitidine or omeprazole for any of the measured variables. There were differences (P<0.05) in tCO₂, SID, PCV, TP, LA and electrolyte concentrations relative to sampling time. However, these differences were attributable to the physiological pressures associated with acute exercise and not an effect of the medication. It was concluded that ranitidine or omeprazole did not alter plasma tCO₂ concentration.

> Key Words: ranitidine • omeprazole • total carbon dioxide • strong ion difference • acid–base status



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EFFECT OF OMEPRAZOLE ON MARKERS OF PERFORMANCE IN GASTRIC ULCER-FREE STANDARDBRED HORSES

J.M. McKEEVER, K.H. McKEEVER, J.M. ALBEIRCI, M.E. GORDON and H.C. MANSO

Reasons for performing study: A large percentage of performance horses develop gastric ulcers and many of those horses are treated with omeprazole. Unfortunately, no data have been published on the effects of the drug on markers of performance in animals without ulcers.

Hypothesis: Omeprazole would alter markers of aerobic and anaerobic performance.

Methods: Ten unfit, healthy, ulcer free, Standardbred mares were administered either control (CON; oral apple sauce, 20 ml) or omeprazole (OP; oral paste, 4 mg/kg bwt s.i.d.) in a random crossover fashion with the investigators blind to the treatment. Treatments were administered for 7 days prior to performing an incremental exercise test (GXT) on a high-speed treadmill. Endoscopic examinations were performed just prior to the trial to verify that the mares were ulcer-free. During the GXT, the mares ran on a treadmill up a 6% grade to measure maximal oxygen consumption (VO_{2max}), run time (RT), velocity at VO_{2max}, maximal velocity (V_{max}), packed cell volume (PCV), plasma lactate concentration (LA) and plasma protein concentration (TP). Measurements were recorded at rest, at the end of each 1 min step of the GXT and at 2 and 5 min post GXT. Data were analyzed using ANOVA for repeated measures and t tests for paired comparisons.

Results: There was no effect (P>0.05) of omeprazole on VO_{2max} ; velocity at VO_{2max} ; RT; V_{max} ; 2 min recovery plasma LA. Nor were there any changes (P>0.05) in the relationship between treadmill speed and VO2, PCV, TP, or plasma LA.

Conclusions: Omeprazole does not appear to improve physiological markers of performance in healthy, ulcer free horses.

Potential relevance: These data may benefit various authorities responsible for deciding administration and timing policies of omeprazole as well as clinicians and horse owners.

Key Words: horse • *omeprazole* • *gastric ulceration* • *performance markers*



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EFFECT OF PHENYLBUTAZONE ON THE HAEMODYNAMIC, ACID-BASE AND EICOSANOID RESPONSES OF HORSES TO SUSTAINED SUBMAXIMAL EXERTION

K.W. HINCHCLIFF, K.H. McKEEVER and W.W. MUIR III

The systemic haemodynamic and acid-base effects of the administration of phenylbutazone (4.4 mg kg⁻¹ intravenously) to standing and running horses were investigated. Phenylbutazone, or a placebo, was administered to each of six mares either 15 minutes before, or after 30 minutes of a 60-minute submaximal exercise test which elicited heart rates approximately 55 percent of maximal, and to the same horses at rest. The variables examined included the cardiac output, heart rate, systemic and pulmonary arterial pressures, right atrial and right ventricular pressures, and arterial and mixed venous blood gases and pH. Serum sodium, potassium and chloride concentrations, and plasma thromboxane B₂, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF_{1 α}), and prostaglandin E₂ (PGE₂) concentrations were measured in separate studies using similar protocols in the same horses. Running produced increases in heart rate, cardiac output, mean arterial and right ventricular pressure, and decreases in total peripheral resistance. The acid:base responses to exertion were characterized by respiratory alkalosis. Exertion did not significantly influence plasma 6-keto-PGF_{1a} or PGE₂ concentrations but plasma thromboxane B2 concentrations were increased significantly by 60 minutes of exertion in the untreated horses. This exercise-induced increase in plasma thromboxane B₂ concentration was inhibited by the previous administration of phenylbutazone, but phenylbutazone did not produce detectable changes in systemic haemodynamic or acidbase variables in either standing or running horses.



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EFFECT OF SUCRALFATE ON TOTAL CARBON DIOXIDE CONCENTRATION IN HORSES SUBJECTED TO A SIMULATED RACE TEST

T.J. CALTABILOTA, J.G. MILIZIO, S. MALONE, J.D. KENNEY and K.H. McKEEVER

The purpose of this study was to test the hypothesis that sucralfate, a gastric ulcer medication, would alter plasma concentrations of total carbon dioxide (tCO₂), lactate

(LA), sodium (Na⁺), potassium (K⁺), chloride (Cl⁻) and total protein (TP), as well as calculated plasma strong ion difference (SID) and packed cell volume (PCV) in horses subjected to a simulated race test (SRT). Six unfit Standardbred mares (~520 kg, 9-18 years) were used in a randomized crossover design with the investigators blinded to the treatment given. The horses were assigned to either a control (40–50 mL apple sauce administered orally (PO)) or a sucralfate (20 mg/kg bodyweight dissolved in 40-50 mL apple sauce administered PO) group. Each horse completed a series of SRTs during which blood samples were taken via jugular venipuncture at five sampling intervals (prior to receiving treatment, prior to SRT, immediately following exercise, and at 60 and 90 min post-SRT). During the SRTs, each horse ran on a treadmill fixed on a 6% grade for 2 min at a warm-up speed (4 m/s) and then for 2 min at a velocity predetermined to produce VO_{2max}. Each horse then walked at 4 m/s for 2 min to complete the SRT. Plasma tCO₂, electrolytes, LA, and blood PCV and TP were analyzed at all intervals. No differences (P>0.05) were detected between control and sucralfate for any of the measured variables. There were differences (P<0.05) in tCO2, SID, PCV, TP, LA and electrolyte concentrations relative to sampling time. However, these differences were attributable to the physiological pressures associated with acute exercise and were not an effect of the medication. It was concluded that sucralfate did not alter plasma tCO₂ concentration in this study.

Key Words:

sucralfate • equine • gastric ulcer syndrome • total carbon dioxide • strong ion difference • acid-base status



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EFFECT OF TRYPTOPHAN AND OF GLUCOSE ON EXERCISE CAPACITY OF HORSES

J.W. FARRIS, K.W. HINCHCLIFF, K.H. McKEEVER, D.R. LAMB and D.L. THOMPSON

We hypothesized that central fatigue may have a role in limiting the endurance capacity of horses. Therefore, we tested the effect of infusing tryptophan and/or glucose on endurance time and plasma concentrations of free tryptophan and other substrates thought to affect tryptophan uptake into the brain of seven mares (3-4 yr of age, 353-435 kg) that ran on a treadmill at 50% of maximal O2 consumption to fatigue. With use of a counterbalanced crossover design, the horses were infused with tryptophan (100 mg/kg in saline solution) or a similar volume of saline solution (placebo) before exercise. During exercise, horses received infusions of glucose (2 g/min, 50% wt/vol) or a similar volume of saline. Thus the treatments were 1) tryptophan and glucose (T & G), 2) tryptophan and placebo (T & P), 3) placebo and glucose (P & G), and 4) placebo and placebo (P & P). Mean heart rate, hematocrit, and concentration of plasma total solids before and during exercise were similar for all trials. Mean time to exhaustion was reduced (P<0.05) for T & P and T & G compared with P & P [86.1 ± 6.9 and 87.1 ± 6.8 vs. 102.3 ± 10.3 (SE) min], whereas endurance for P & G (122.4 ± 11.9 min) was greater than for all other trials (P<0.05). Compared with non-tryptophan trials, during the tryptophan trials plasma prolactin increased (P<0.05) nearly threefold before exercise and almost twofold early in exercise. Muscle glycogen concentrations were reduced (P<0.05) below pre-exercise values in the P & G and P & P trials only. However, glucose infusions (P & G) did not affect (P>0.05) concentrations of plasma free fatty acids or ratios of branched-chain amino acids to free tryptophan. In conclusion, tryptophan infusion reduced endurance time, which was consistent with the central fatigue hypothesis. The failure of glucose infusion to alleviate the effects of tryptophan and the absence of significant muscle glycogen reduction in the tryptophan trials suggest that the early onset of fatigue in the tryptophan trials is not due to a lack of readily available substrate.

> Key Words: central fatigue • oxygen consumption • serotonin • prolactin • branched-chain amino acids



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EFFECTS OF CLENBUTEROL ON HORSES

S.D. DRAKE, L.E. HANSEN, C. HARRIS, W.C. LEWIS, E. MILLER, B. MORANVILLE, M. BLYZKA, W.G. BERGEN, K.H. McKEEVER, G.J. HAUSMAN and M.V. DODSON

Clenbuterol was intended as a treatment for respiratory diseases in horses, but has been used in multiple species, including humans, for its repartitioning of fat to lean effects (free fatty acids are released from adipose tissue to be used by tissues of higher priority). In the horse industry clenbuterol application is restricted to the treatment of chronic obstructive pulmonary disease and reactive airway disease (heaves). Negative effects of clenbuterol exposure include a decrease in maximum oxygen intake and increased muscle fatigue upon exercise. As a result of these and other negative effects, clenbuterol remains strictly controlled by the US Food and Drug Administration.

 $\label{eq:keyWords:} \textit{Key Words:} \\ \beta_2 \textit{-adrenergic agonist} \bullet \textit{repartitioning, equine} \bullet \textit{bronchodilator} \\$



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EFFECTS OF COCAINE ON INCREMENTAL TREADMILL EXERCISE IN HORSES

K.H. MCKEEVER, K.W. HINCHCLIFF, D.F. GERKEN and R.A. SAMS

Four mature horses were used to test the effects of two doses (50 and 200 mg) of intravenously administered cocaine on hemodynamics and selected indexes of performance [maximal heart rate (HR_{max}), treadmill velocity at HR_{max}, treadmill velocity needed to produce a blood lactate concentration of 4 mmol/l, maximal mixed venous blood lactate concentration, maximal treadmill work intensity, and test duration] measured during an incremental treadmill test. Both doses of cocaine increased HR_{max} approximately 7% (P<0.05). Mean arterial pressure was 30 mmHg greater (P<0.05) during the 4- to 7-m/s steps of the exercise test in the 200-mg trial. Neither dose of cocaine had an effect on the responses to exertion of right atrial pressure, right ventricular pressure, or maximal change in right ventricular pressure over time. Maximal mixed venous blood lactate concentration increased 41% (P<0.05) with the 50-mg dose and 75% (P<0.05) with the 200-mg dose during exercise. Administration of cocaine resulted in decreases (P<0.05) in the treadmill velocity needed to produce a blood lactate concentration of 4 mmol/l from 6.9 ± 0.5 and 6.8 ± 0.9 m/s during the control trials to 4.4 \pm 0.1 m/s during the 200-mg cocaine trial. Cocaine did not alter maximal treadmill work intensity (P>0.05); however, time to exhaustion increased by approximately 92 s (15%; P<0.05) during the 200-mg trial.



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EFFECTS OF QUERCETIN ON EXERCISE POTENTIAL AND EXERCISE-INDUCED CYTOKINES IN THE HORSE

J. BALDASSARI, W.C. FRANKE, D.W. HOROHOV, A. BETANCOURT and K.H. McKEEVER

The goal of this experiment was to test the hypothesis that quercetin would alter markers of athletic performance as well as markers of exercise-induced inflammation. Six healthy, unfit Standardbred mares (~500 kg, age 4-5 years) were assigned to one of two treatments (quercetin or water) in an experiment conducted in a crossover fashion. The horses were dosed via a nasogastric tube twice a day (08:00 and 20:00) with either 6 g of quercetin in 2 l tap water or 2 l water for 3.5 d. Two hours after the seventh and final dose, a graded exercise test (GXT) was run. During the test the treadmill remained at a fixed 6% grade. At t=0 the treadmill was started at a speed of 4 m/s. Each following minute the treadmill speed was increased by 1 m/s, up to a maximum speed of 11 m/s. Blood and muscle samples were collected before the initial dosing, before exercise and after the GXT to measure markers of inflammation. There was a significant (P<0.10) increase in run time to fatigue when the horses were dosed with quercetin. The VO₂ recovery time was shortened significantly when compared to water-treated trials. There were exerciseinduced increases (P<0.10) in haematocrit and the plasma concentrations of total protein, glucose, and lactate as well as expression of mRNA for TNF- α , IL-1, interferon- γ , granzyme-B (GrB) in blood. However, quercetin only affected (P<0.10) the expression of mRNA for GrB. Intramuscular levels of IL-1 and GrB also increased significantly with exercise, but there was no effect (P>0.10) from quercetin treatment. This study provides evidence that quercetin could be useful in enhancing exercise performance, although the mechanism for this enhancement is unclear.

> *Key Words: equine* • *exercise* • *inflammation* • *muscle* • *cytokine* • *quercetin*



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EPHEDRA INCREASES VO₂, IMPAIRS THERMOREGULATION AND CARDIOVASCULAR FUNCTION, BUT DOES NOT ENHANCE EXERCISE PERFORMANCE

R.P. NORTON, R.A. LEHNHARD, C.F. KEARNS and K.H. McKEEVER

The dietary supplement ephedra is a potent sympathomimetic that was banned by the US Food and Drug Administration in 2003 because of its deleterious effects on cardiovascular function and thermoregulation during exercise. Unfortunately, extracts of ephedra can still be obtained via the internet and are in use worldwide. The horse is the only athletic species other than humans that sweats to thermoregulate and it controls cardiovascular function in a similar fashion. The purpose of this study was to use the horse to examine the acute effects of ephedra (Ma Huang) to investigate on markers of performance as well as effects on cardiovascular function and thermoregulation during acute exercise. Six Standardbred mares (~450 kg; 6-12 years of age) were used in a crossover design consisting of an ephedra (Ma Huang containing 8% ephedra alkaloid) and control (applesauce) group. All horses performed an incremental graded exercise test (GXT) at a 6% fixed grade to measure oxygen uptake (VO_2) , run time, velocity at VO_{2max}, maximal velocity, recovery time, haematocrit, total plasma protein concentration, heart rate, right ventricular pressure (RVP), pulmonary arterial pressure (PAP), rectal temperature and recovery. Measurements were recorded at rest, during exercise and post 2 and 5 min recovery. There was a difference (P<0.05) in pre-exercise haematocrit but not in any other haematocrit or plasma protein sampling intervals. VO₂ was greater (P<0.05) for the ephedra group before exercise, at each step of the GXT, at VO_{2max}, and during recovery compared to the control group. Recovery time was significantly different, but run time was not (P>0.05). Heart rate was elevated (P<0.05) at 2 and 5 min recovery in horses administered ephedra. Significant differences were observed for RVP and PAP and rectal temperature during recovery. Recovery score (sweating response, respiration rate, behavior) was altered (P<0.05) by ephedra administration. These data suggests an increase in energy expenditure and thermogenesis when horses consume ephedra. However, markers of performance (run time, velocity at VO_{2max}, and maximal velocity completed) were not altered by ephedra administration.

> Key Words: ephedra • horse • haemodynamics



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EXTRARENAL EFFECTS OF FUROSEMIDE

K.W. HINCHCLIFF, K.H. McKEEVER and W.W. WUIR III

The potent diuretic activity of furosemide is widely acknowledged, but the fact that furosemide also has considerable extrarenal activity is often overlooked. The principal extrarenal effects of furosemide include decreases in pulmonary blood volume and reductions in cardiac filling pressures. The hemodynamic effects of furosemide appear to be due to an increase in venous compliance and a decrease in plasma volume. The hemodynamic effects of furosemide are apparent during exertion in horses as decreases in right ventricular filling pressures, pulmonary artery pressure, and stroke volume and increases in heart rate. Furosemide decreases body weight, coincident with a reduction in plasma volume, and induces a metabolic alkalosis. The individual effect o each of these changes on athletic performance is unclear, although the net effect is apparently to improve athletic performance in certain circumstances.



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FEED INTAKE, BODY WEIGHT, BODY CONDITION SCORE, MUSCULATION, AND IMMUNOCOMPETENCE IN AGED MARES GIVEN EQUINE SOMATOTROPIN

K. MALINOWSKI, R.A. CHRISTENSEN, A. KONOPKA, C.G. SCANES and H.D. HAFS

Sixteen 20- to 26-yr-old mares were given 0, 6.25, or 12.5 mg/d equine somatotropin (eST) to determine whether aged mares respond to ST with changes in feed intake, body weight, body condition score (based mostly on fat cover), or immunocompetence. Neither dry matter intake, body weight, nor body condition scores were altered during the 6 wk of eST injection. However, based on photographs taken to evaluate musculation before and after treatment (scores 0 to 4), mares given eST developed greater (P<0.07) muscle definition (1.8 \pm 0.6 and 2.5 \pm 0.6 for 6.25 and 12.5 mg eST/d, respectively) than control mares (0.7 ± 0.4) . Total circulating leukocytes increased (P<0.05) in both of the eST-treated groups during the 6-wk injection period, caused by an increase (P<0.05) in granulocytes. Lymphocyte numbers were not altered. Granulocyte oxidative burst activity was not altered by eST treatment. Although lymphocyte proliferative responses to phytohemagglutinin, pokeweed mitogen, or lipopolysaccharide were not altered during the treatment period, lymphocyte proliferation in response to phytohemagglutinin and pokeweed mitogen increased twofold in eST-treated horses at 2 wk after eST treatment. In overview, the increased musculation and the increase in granulocyte numbers in mares given eST suggest that eST supplementation may improve the health and well-being of aged mares.

> *Key Words: horses* • *somatotropin* • *immunocompetence* • *leukocytes*



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FLUID ADMINISTRATION ATTENUATES THE HAEMODYNAMIC EFFECT OF FRUSEMIDE IN RUNNING HORSES

K.W. HINCHCLIFF and K.H. McKEEVER

The effect of blood volume repletion after frusemide administration on the right atrial and pulmonary artery pressure responses of horses to exercise has not been reported. We examined right atrial and pulmonary artery pressure and plasma atrial natriuretic peptide concentration (ANP) responses to an incremental exercise test in 6 Standardbred mares. Horses were treated, in a 3 way cross over design, with isotonic saline, frusemide (1 mg/kg bwt, i.v.), and frusemide followed 3 h later by lactated Ringer's solution (12 ml/kg bwt, i.v.). Three and a half hours after saline or frusemide administration the horses completed a standard exercise test. Frusemide significantly affected the right atrial and pulmonary artery pressure and ANP responses to exercise. Fluid administration decreased plasma total protein concentrations at rest and during running and abolished the effects of frusemide on the haemodynamic and ANP responses to exercise. These results suggest that the haemodynamic effect of frusemide in running horses is mediated, in large part, by a reduction in plasma and blood volume.

> *Key Words: horse* • *atrial natriuretic peptide* • *lasix* • *exercise-induced pulmonary haemorrhage*



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FRUSEMIDE

K.W. HINCHCLIFF and K.H. McKEEVER

Frusemide is administered to large numbers of North American racehorses because of its putative prophylactic effect on exercise induced pulmonary haemorrhage (EIPH). Two issues central to the use of frusemide in performance horses remain to be resolved: 1) Does frusemide reduce the incidence or severity of EIPH? and 2) does frusemide alter athletic performance or the capacity for muscular work, separate from any effect on EIPH? These issues are discussed, as is the reasoning that EIPH is, at least in part, a consequence of exertion induced high vascular transmural pressures and that reduction of these pressures by frusemide is the mechanism by which frusemide reduces the incidence or severity of EIPH. It is concluded that, on balance, it appears unlikely that frusemide reduces the incidence of EIPH, although it may reduce the severity of haemorrhage. The evidence that frusemide is ergogenic is evaluated and a potential mechanism for an effect of frusemide on athletic capacity, the frusemide induced reduction in body weight, is discussed.

Key Words: horse • frusemide • exercise • performance • exercise induced pulmonary haemorrhage



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FRUSEMIDE AND WEIGHT CARRIAGE ALTER THE ACID:BASE RESPONSES OF HORSES TO INCREMENTAL AND TO BRIEF INTENSE EXERTION

K.W. HINCHCLIFF and K.H. McKEEVER

We speculated that frusemide would attenuate the acidosis associated with intense exertion, and that weight carriage would mitigate this effect. Therefore, in each of 2 experiments we measured pulmonary artery and systemic arterial blood pH, PCO₂, PO₂ and pulmonary artery temperature in 9 horses during exertion on a treadmill after each of 3 treatments. The treatments were: 1) injection of saline solution (C), 2) injection of frusemide (1 mg/kg bwt, i.v. 4 h before running) (FU) and 3) injection of frusemide (F) as for FU and the horses carried weight equal to that lost in the 4 h after frusemide administration (FL). In Experiment I the horses performed an incremental exercise test of 90 s at each of 4, 7 and 9 m/s (4° incline). In Experiment 2, the horses ran on a treadmill inclined at 4° for 3 min at 3 m/s, at approximately 120% VO2max for 2 min, and 3 m/s for 5 min. Blood samples for measurement of blood pH, PCO₂, and PO₂ were collected into heparinized glass syringes. Values were adjusted for changes in pulmonary artery blood temperature. Data were analyzed using a 2-way repeated measures ANOVA. There was a consistent pattern in both experiments characterized by a mild alkalosis or mitigated acidosis, compared to C, at rest and during exercise and recovery after FU (P<0.05). Weight carriage blunted or inhibited the effect of frusemide during exercise and recovery. In experiment 2, at the end of the sprint, FU treatment resulted in a less severe acidosis than did C or FL: mixed venous pH for C, FU, and FL at the end of the sprint were 6.93, 6.98 and 6.93, respectively (P interaction=0.038), mixed venous base excess was -12.4, -9.91, and -11.8 mmol/l, respectively, and arterial base excess of -14.4, -12.7 and -14.1 mmol/l (P interaction=0.006) respectively. These effects persisted into recovery. A similar pattern of responses occurred during the incremental exercise test, with weight carriage offsetting the alkalinizing effect of frusemide. We conclude that frusemide attenuates the acidosis of intense exertion and that carriage of weight prevents this effect.

> *Key Words: horse* • *blood pH* • *bicarbonate* • *base excess* • *acidosis* • *alkalosis*



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FUROSEMIDE MAGNIFIES THE EXERCISE-INDUCED ELEVATION OF PLASMA VASOPRESSIN CONCENTRATION IN HORSES

K.H. McKEEVER, K.W. HINCHCLIFF, J.L. COOLEY and D.R. LAMB

The purpose of this study was to test the hypothesis that furosemide administration before exercise would cause greater increases in plasma arginine vasopressin (avp) concentration in exercising horses than exercise alone. Six adult, clinically normal, unfit mares underwent three randomly ordered 60 minute standard exercise tests on an equine treadmill to examine the effect of furosemide administration on plasma AVP concentration. In one trial, furosemide (1 mg kg⁻¹) was infused four hours before exercise (fur-4) and a placebo (10 ml saline) was infused two minutes before exercise; in another trial the placebo was infused four hours before exercise and drug was infused two minutes before exercise (fur-2); in a third trial a placebo was infused four hours and two minutes before exercise (con). During the treadmill test each mare ran up a fixed 4° incline for one hour at a speed previously determined to produce a heart rate of 65 per cent of each horse's maximum heart rate. Venous blood samples were collected at rest in the stall, immediately before exercise while standing on the treadmill, and at 15 minute intervals during the treadmill test. Plasma AVP concentration was measured by radioimmunoassay. In the con trial, plasma avp concentration increased 561 per cent (P<0.05) from 6. $\cdot 3 \pm 1.0$ pg/ml (mean \pm se) at rest to 38.8 ± 12.8 pg/ml at the end of the 60 minute run. During the fur-2 trial, avp increased 1185 percent (P<0.05) from 5.9 ± 1.7 pg/ml to 75.8 \pm 17.7 pg/ml. During the fur-4 trial, AVP increased 3624 per cent (P<0.05) from 3.3 ± 0.5 pg/ml to 122.9 ± 33.7 pg/ml at the end of the exercise. These data demonstrate that furosemide significantly enhances the avp response to submaximal exercise in the horse.



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FUROSEMIDE REDUCES ACCUMULATED OXYGEN DEFICIT IN HORSES DURING BRIEF INTENSE EXERTION

K.W. HINCHCLIFF, K.H. MCKEEVER, W.W. MUIR III and R.A. SAMS

We theorized that furosemide-induced weight reduction would reduce the contribution of anaerobic metabolism to energy expenditure of horses during intense exertion. The effects of furosemide on accumulated O_2 deficit and plasma lactate concentration of horses during high-intensity exercise were examined in a three-way balance randomized crossover study. Nine horses completed each of three trials: 1) a control (C) trial, 2) a furosemide-unloaded (FU) trial in which the horse received furosemide 4 h before running, and 3) a furosemide weight-loaded (FL) trial during which the horse received furosemide and carried weight equal to the weight lost after furosemide administration. Horses ran for 2 min at ~120% maximal O_2 consumption. Furosemide (FU) increased O_2

consumption (ml·2 min⁻¹·kg⁻¹) compared with C (268 ± 9 and 257 ± 9, P<0.05), whereas FL was not different from C (252 ± 8). Accumulated O₂ deficit (ml O₂ equivalents/kg) was significantly (P<0.05) lower during FU (81.2 ± 12.5), but not during FL (96.9 ± 12.4), than during C (91.4 ± 11.5). Rate of increase in blood lactate concentration (mmol·2 min⁻¹·kg⁻¹) after FU (0.058 ± 0.001), but not after FL (0.061 ± 0.001), was significantly (P<0.05) lower than after C (0.061 ± 0.001). Furosemide decreased the accumulated O₂ deficit and rate of increase in blood lactate concentration of horses during brief high-intensity exertion. The reduction in accumulated O₂ deficit in FU-treated horses was attributable to an increase in the mass-specific rate of O₂ consumption during the high-intensity exercise test.

Key Words: weight carriage • *locomotion* • *energy expenditure*



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FUROSEMIDE-INDUCED CHANGES IN PLASMA AND BLOOD VOLUME OF HORSES

K.W. HINCHCLIFF, K.H. McKEEVER and W.W. MUIR III

The effect of furosemide administration (1mg/kg body weight, i.v.) on plasma and blood volumes in 6 intact and 4 splenectomized horses was measured using Evans blue dye dilution, hematocrit, and hemoglobin and plasma total solids concentrations. Body weight decreased by 33.6 ± 3.3 and 33.7 ± 0.8 g/kg 4h after furosemide administration to intact and splenectomized mares, respectively. Plasma volume, estimated by Evans blue dye dilution, was reduced by $8.3 \pm 3.3\%$ (mean \pm SE) 4h after furosemide administration. The reduction in plasma volume was first detectable 5-10 min after furosemide administration administration and was greatest 15-30 min ($13.0 \pm 0.8\%$) after dosing. This study demonstrates that furosemide produces significant and rapid reductions in plasma volume in horses. These decreases in plasma volume only partially resolve 4h after furosemide administration.



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HEMODYNAMIC EFFECTS OF ATROPINE, DOBUTAMINE, NITROPRUSSIDE, PHENYLEPHRINE, AND PROPRANOLOL IN CONSCIOUS HORSES

K.W. HINCHCLIFF, K.H. McKEEVER and W.W. MUIR III

The authors investigated the cardiovascular effects of low doses of nitroprusside, dobutamine, and phenylephrine and a β -adrenergic blocking dose of propranolol in conscious, healthy horses with and without prior atropine administration. A parasympathetic blocking dose of atropine produced significant increases in heart rate and arterial pressures, and decreased stroke volume, ejection fraction, pulse pressure, and right-ventricular end-diastolic pressure and volume. Cardiac output was not changed by atropine administration. Nitroprusside reduced arterial pressures to a greater extent in atropinized horses but increased heart rate in both atropinized and non-atropinized horses. Dobutamine increased mean arterial pressure in both non-atropinized and atropinized horses but increased heart rate, diastolic arterial pressure, and systemic vascular resistance only in atropinized horses. Propranolol did not affect any of the hemodynamic variables that were measured. Phenylephrine, in the presence of β adrenergic blockade, increased mean arterial pressure and reduced cardiac output. This study showed that low doses of nitroprusside, dobutamine, and phenylephrine produce significant hemodynamic effects in conscious, healthy horses and that these effects are modified by prevailing parasympathetic tone.



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LOW DOSE EXOGENOUS ERYTHROPOIETIN ELICITS AN ERGOGENIC EFFECT IN STANDARDBRED HORSES

K.H. McKEEVER, J.M. AGANS, S. GEISER, P.J. LORIMER and G.A. MAYLINS

Reasons for performing study: Recombinant human erythropoietin (rhuKPO) causes an increase in red blood cell production and aerobic capacity in other species; however, data are lacking on effects in the horse.

Hypothesis: This study tested the hypothesis that rhuEPO administration would alter red cell volume (RCV), aerobic capacity (VO_{2max}) and indices of anaerobic power.

Methods: Eight healthy, unfit mares accustomed to the laboratory and experimental protocols were randomly assigned to either a control (CON, n=4; 3 ml saline 3 times/week for 3 weeks) or EPO group (EPO, n=4, 50 iu/kg bwt rhuEPO/3 ml saline 3 times/week for 3 weeks). Exercise tests (GXT) were performed on a treadmill (6% incline), 1 week before and 1 week after treatment. The GXT started at 4 m/s, with a 1 m/s increase every 60 sec 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 packed cell volume (PCV), haemoglobin concentration (Hh), blood lactate concentration (LA) and plasma protein concentration (TP). Plasma volume (PV) was measured using Evans Blue dye. Blood volume (HV) and RCV were calculated using PCV 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 PCV ($37 \pm 2 \text{ vs. } 51 \pm 2$) and Hb (37%). RCV (26%) and VO_{2max} (19%)increased, but BV did not change (P>0.05) due to decreased PV (-11%, P<0.05). There was a significant increase in velocity at VO_{2max} and LA_{peak} for horses treated with rhuEPO and substantial decrease (P<0.05) in VO₂ recovery time when the pretreatment GXT was compared to the post treatment GXT. No differences (P<0.05) were detected for TP, VLA₄, run time or V_{max}.

Conclusions: Low dose rhuEPO administration increases RCV and aerobic capacity without altering anaerobic power.

Potential relevance: This study demonstrates that rhuEPO enhances aerobic capacity and exercise performance, a question relevant to racing authorities.

Key Words: horse • *erythropoietin* • *blood doping* • *aerobic capacity*



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PHARMACOLOGIC INTERACTION OF FUROSEMIDE AND PHENYLBUTAZONE IN HORSES

K.W. HINCHCLIFF, K.H. McKEEVER, W.W. MUIR III and R.A. SAMS

The effect of premedication with phenylbutazone on systemic hemodynamic and diuretic effects of furosemide was examined in 6 healthy, conscious, mares. Mares were instrumented for measurement of systemic hemodynamics, including cardiac output and pulmonary arterial, systemic arterial, and intracardiac pressures, and urine flow. Each of 3 treatments was administered in a randomized, blinded study; furosemide (1 mg/kg of body weight, IV) only, phenylbutazone (8.8 mg/kg, PO, at 24 hours and 4.4 mg/kg, IV, 30 minutes before furosemide) and furosemide, or 0.9% NaCl. Phenylbutazone administration significantly attenuated, but did not abolish, the diuretic effect of furosemide. Phenylbutazone completely inhibited the immediate effect of furosemide on cardiac output, stroke volume, total peripheral resistance, and right ventricular peak pressure. Premedication with phenylbutazone did not inhibit equally the diuretic and hemodynamic effects of furosemide, indicating that some of furosemide's hemodynamic effects are mediated by an extrarenal activity of furosemide.



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PHENYLBUTAZONE BLOCKS THE CYTOKINE RESPONSE FOLLOWING A HIGH-INTENSITY INCREMENTAL EXERCISE CHALLENGE IN HORSES

R.A. LEHNHARD, A.A. ADAMS, A. BETANCOURT, D.W. HOROHOV, N.R. LIBURT, J.M. STRELTSOVA, W.C. FRANKE and K.H. McKEEVER

This study tested the hypothesis that phenylbutazone would block the exercise-induced increase in cytokine markers of inflammation in blood. Blood samples were obtained from unfit Standardbred mares (age 10 ± 4 years, 500 kg) before and after three different trials (standing control (CON), n=9; exercise with phenylbutazone (EX-bute), n=9; and exercise with water, n=9). Comparisons were made for data collected in three trials, one where each horse underwent an incremental exercise test (graded exercise test (GXT)) where they were administered water as a placebo, a GXT following phenylbutazone administration (2 g given orally 2 h before the GXT) or standing parallel control where they stood quietly in stalls. During the GXT, horses ran on a treadmill (1 m/s increases each min until fatigue, 6% grade). Blood samples were obtained 30 min before exercise, immediately after exercise and at 0.5, 1, 2, 4 and 24 h post-GXT or at matched time points during the parallel control trials. Samples were analyzed using real-time PCR for measurement of mRNA expression of interferon- γ (IFN- γ), tumour necrosis factor- α $(TNF-\alpha)$ and interleukin (IL)-6 in samples collected during all three trials, and for IL-1 and IL-10 in samples collected for the CON and EX-bute trials. Data were analyzed using ANOVA for repeated measures, and where appropriate, post hoc separation of means utilized the Student-Newman-Keuls test. The null hypothesis was rejected when P<0.05. There were no changes (P>0.05) in IL-1, IL-6, IFN- γ or TNF- α during CON or following phenylbutazone administration. During the water trial, exercise resulted in significant increases in IFN- γ , IL-1 and TNF- α . It was concluded that high-intensity exercise results in a transient increase in the expression of inflammatory cytokines in blood that is blocked when phenylbutazone is administered to horses.

> Key Words: horse • cytokines • non-steroidal anti-inflammatory drugs • exercise • equine • muscle



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PULSATILE RELEASE OF SOMATOTROPIN RELATED TO MEAL FEEDING AND SOMATOTROPIN RESPONSE TO SECRETAGOGUES IN HORSES

R.A. CHRISTENSEN, K. MALINOWSKI, C.G. SCANES and H.D. HAFS

Our goal was to establish a time of day and(or) interval from feeding that would avoid the refractory period after a somatotropin (ST) surge and optimize the responsiveness of horses to ST secretagogues. Two experiments were conducted with eight geldings conditioned to consume grain at 0800 and 1600 daily. In Exp. 1, during a 24-h period, these geldings averaged 3.2 ± 0.3 pulses of ST with peak amplitude of 4.2 ± 0.4 ng/mL, pulse duration of 55 ± 6 min, and interpeak interval of 400 ± 57 min. No ST peaks occurred within 2 h after either grain feeding. In Exp. 2, eight geldings were given 50 micrograms of ST-releasing factor (STRF) at 0800. Two geldings that had a pulse of ST between 0700 and 0800 failed to respond to STRF, but the other six responded with a pulse of ST at 37 ± 3 min; peak amplitude was 4.6 ± 2.2 ng/mL and duration was 123 ± 25 min. Experiments 3 and 4 were with mares aged 20 to 26 yr and conditioned to be fed grain at 0800 daily. In Exp. 3, blood was sampled for 8 h beginning at 0500. Seven of the eight mares had a ST pulse in progress at 0500. Five additional pulses were detected, all from 0740 to 0940, but none from 0600 to 0700 or from 1000 to 1300. In Exp. 4, four of the same eight mares were given 50 micrograms of STRF at 0700 and the other four at 1300. Three of the four treated at 0700 and all four treated at 1300 responded to STRF with ST peaks at 20 \pm 5 min; peak amplitude was 12.7 \pm 9.5 ng/mL and duration was 69 \pm 6 min. In Exp. 5, nine mares aged 20 to 26 yr were fed grain at 0800 and 1600 as in Exp. 1 and 2 and given a nonpeptidal ST secretagogue (STS, Merck L-163,255) i.v. at 0, 1, or 5 mg/kg (n=3 mares/dose) at 1300. No mare had a pulse of ST during the 1 h before treatment. All six mares given STS responded with ST pulses. The ST responses to STS at 1 and 5 mg/kg did not differ (P>0.05); time to ST peak was 35 ± 4 min, pulse amplitude was 24.0 \pm 6.3 ng/mL, and pulse duration was 100 \pm 9 min. We conclude that mares and geldings fed grain once or twice daily usually have a period of 2 to 5 h after feeding with no ST pulses. When horses are fed grain at 0800, one may give a ST secretagogue at 1300 to avoid a refractory period and improve the probability of an ST response.



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THERAPEUTIC CLENBUTEROL TREATMENT DOES NOT ALTER CA²⁺ SENSITIVITY OF PERMEABILIZED FAST MUSCLE FIBRES FROM EXERCISE TRAINED OR UNTRAINED HORSES

D.R. PLANT, C.F. KEARNS, K.H. McKEEVER and G.S. LYNCH

Clenbuterol is a β 2-adrenoceptor agonist primarily used for treating bronchospasm and alleviating the symptoms of chronic obstructive pulmonary disease (COPD) in the horse. In other species (rats, mice, sheep, and cattle), chronic high doses of clenbuterol (typically in the milligram per kilogram body weight range) has been shown to cause a muscle directed protein anabolic response. Clenbuterol can also modify muscle fibre composition and therefore potentially affect muscle function. This has implications for the performance of exercising horses being treated with therapeutic doses of clenbuterol (typically in the microgram per kilogram body weight range) for bronchospasm or COPD. It is not known whether clenbuterol treatment affects muscle fibre function in horses. The purpose of this study was to examine the effects of a therapeutic dose of clenbuterol, with and without exercise, on the contractile activation characteristics of single membrane permeabilized fibres prepared from muscle biopsies. We tested the hypothesis that therapeutic treatment with clenbuterol would not affect muscle fibre function. Unfit Standardbred mares were treated for 8 weeks with; clenbuterol (2.4 µg/kg twice/day, 5 days/week) plus exercise (20 min at 50%=VO_{2max} 3 d/wk; CLENEX), clenbuterol only (CLEN), or exercise only (EX). Muscle biopsies were taken from the gluteus medius muscle before and after treatment and stored in a glycerol-based solution to prepare permeabilized muscle fibres. The force-pCa relationship for fibres from CLEN horses was steeper (P<0.05) indicative of greater cooperative interactions within the thin filament, however, fibre sensitivity to Ca^{2+} was unchanged. In contrast, the steepness of the force-pCa relationship was not changed in fibres from EX and CLENEX horses and Ca²⁺ sensitivity was also unaffected. Rigor force, activation in the absence of ATP, was not affected by any treatment indicating an approximately equivalent number of participating cross-bridges during activation. The results indicate that a therapeutic dose of clenbuterol to Standardbred horses does not affect the Ca²⁺-activated contractile characteristics of isolated muscle fibres.



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