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Effect of sodium and potassium chloride supplementation on serum electrolyte concentrations, water intake, body weight and performance of endurance horses.

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Introduction

We hypothesized that oral administration of a high dose (HD) of sodium chloride (NaCl) and potassium chloride (KCl) to endurance horses would differentially increase water intake, attenuate body weight loss and improve performance when compared to a low dose (LD).

Material and methods

To test this hypothesis, a randomized, blinded, cross-over study was conducted on 8 horses participating in 2 x 50 mile rides (same course, 28 days apart). Thirty minutes before and at 25 miles of the first ride 4 horses received orally 0.22 g/kg NaCl and 0.074 g/kg KCl (HD); the other 4 received 0.07 g/kg NaCl and 0.02 g/kg KCl (LD). The horses received the alternate treatment in the second ride. Data were analyzed with 2-way ANOVA for repeated measures ($P < 0.05$).

Results

Treatment significantly affected serum [Na], [Cl], [HCO3], pH and water intake, but not serum [K] or body weight. Serum [Na] and [Cl] were significantly higher at 50 miles when horses received HD, but no differences were found in early recovery. Venous [HCO3] and pH were significantly lower throughout the ride and in early recovery when horses received HD. Estimated water intake was significantly greater both at the 25 mile mark and as a cumulative estimate following HD supplementation. No differences between treatments were detected in body weight loss or completion time.

Conclusion

Other than enhancing water intake, supplementation of endurance horses with high doses of NaCl and KCl did not provide any detectable competitive advantage. Furthermore, the elevated serum electrolyte concentrations induced with HD might not be appropriate for endurance horses.

Endurance exercise is associated with increased plasma cardiac troponin I in horses

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Introduction

Endurance exercise is associated with increased plasma cardiac troponin I in horses Cardiac troponin I (cTNI) is an integral regulatory peptide with critical physiologic roles during excitation-contraction coupling in the heart. Although most often utilized as a serum biomarker in the diagnosis of myocardial infarction, other conditions including sepsis, renal failure, and strenuous exercise can result in systemic increases of cTNI in humans. The significance of increased cTNI associated with strenuous exercise is under debate. We sought to determine the following : i) if cTNI increases subsequent to endurance exercise in horses, and ii) if the increase is correlated with performance.

Material and methods

Data were collected at three ride sites during the 2004-2005 American Endurance Ride Conference season. Heparinized plasma for cTNI analysis, and 3 minute electrocardiogram recordings were obtained prior to, at the midpoint, and end of 50 and 100 mile competitions. Finish position, status, and ride times were obtained from the official ride management results.

Results

One horse developed paroxysmal atrial fibrillation (AF) during the study. cTNI from this horse was elevated (0.94 ng/mL) at the time of AF. Of the 321 horses in the study, 96 had complete sets of data for analysis. Pre-competition mean cTNI was 0.02 ng/mL. Endurance exercise was associated with a significant increase in cTNI in both the 100 (P = 0.0007), and 50 mile (P = 0.02) distance groups. Furthermore, when data from both distances were combined, horses that performed poorly (failed to finish competition) had an increased cTNI (P = 0.03). Horses in the 100 mile race that finished in the top 10 did not have significant increases in mean completion cTNI whereas horses that finished worse did.

Conclusion

The clinical implication of increased cTNI in horses during endurance rides deserves further study.

Use of electrolytes during long distance endurance rides about the French experience

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Introduction

To replace electrolytes lost in sweat during endurance competition, riders frequently supplement horses with hypertonic oral electrolyte pastes. Sweat losses in Na+, Cl- and K+ during a 10 hour endurance competition can be grossly estimated respectively to 300g, 550g and 120g. Oral pastes administered to endurance horses usually bring less than 5g of each ion per syringe. Therefore, their utility during long distance endurance rides is questionable.

Material and methods

A prospective and retrospective study was conducted on horses from the French endurance team during international competitions. Since 2002, during the 160 km rides, horses received no oral electrolytes except those contained in food and water. The frequency of metabolic troubles and the performance of the horses (speed, place at the arrival and medals) were compared with those obtained previously when horses were systematically supplemented.

Results

Before 2002, on each international event, at least one horse and most often several horses presented anorexia or stopped voluntary drinking during the ride. In the absence of electrolyte supplementation, no horse stopped drinking or eating; consequently, the horses were considered easier to manage by the riders. The frequency of elimination or withdrawals for metabolic troubles was significantly reduced ($P < 0.05$) since 2002. There was no statistical difference in the performance of the supplemented and non-supplemented horses. From 2002 to 2004, French team horses contributed to get 4 medals without any electrolyte supplementation.

Conclusion

Electrolyte supplementation in well-conditioned horses is not essential during long distance endurance rides.

Thyroid hormone responses to endurance exercise

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Introduction

To assess the effects of prolonged exercise on thyroid gland function, thyroid hormone (TH) concentrations were measured in horses competing in endurance rides as well as during recovery from a 60 km treadmill exercise test.

Material and methods

Concentrations of total thyroxine (T4), free thyroxine (FT4), total triiodothyronine (T3), and free triiodothyronine (FT3) were measured in blood samples collected at the start and shortly after successful completion of endurance rides of 40 (n = 59), 56 (n = 27), 80 (n = 32), and 160 (n = 10) km. Next, T4, T3, and reverse triiodothyronine (rT3) were measured in blood samples collected daily from nine horses that successfully completed a 5-day, 424 km ride. Finally, T4, FT4, T3, and FT3 were measured before and during 72 h of recovery from a 60 km treadmill run. Serum concentrations of the thyroid hormones were determined by radioimmunoassays that have been validated for use on equine sera.

Results

During a single day ride, distance-dependent decreases in T4, FT4, T3, and FT3 occurred with the greatest reductions observed in horses completing 160 km. In horses that completed the multi-day ride, T4, T3, and rT3 progressively decreased over the course of the ride. After completion of 60 km of treadmill exercise, T4, FT4, T3, and FT3 remained lower than pre-exercise values during the initial 24-48 h of recovery.

Conclusion

These data demonstrate that TH concentrations decrease during endurance rides of 80 km or greater and that the magnitude of the decrease increases with ride distance. Furthermore, repeated bouts of endurance exercise lead to progressive decreases in T4, T3, and rT3. Finally, after endurance exercise of only 60 km, TH concentrations remain decreased for up to 48 h. Taken together, these data suggest that endurance exercise results in more rapid tissue utilization of circulating thyroid hormones, but no evidence of inadequate thyroid gland function was found. Displacement of protein bound hormone by increases in other metabolites (e.g, free fatty acids) could be an additional explanation for decreases in T4 and T3.

Changes in selected physiological and laboratory parameters in elite horses competing in a 160 km endurance race

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Introduction

To provide initial data describing changes in physiological and laboratory parameters in elite horses competing at a higher speed, 160 km endurance race under temperate conditions and to compare data between horses that successfully completed the race and those that failed to finish.

Material and methods

Body mass (BM) was measured and veterinary examinations were performed on 36 horses before, during, and at the finish of the 160 km Endurance Nations Cup held in Newmarket on June 27, 2004. In addition, blood samples were collected on the afternoon prior to the race and shortly after completion or elimination from the competition.

Results

Amongst 36 horses participating in the study, 22 completed the race. Twelve horses were eliminated for lameness and two were eliminated for a persistently elevated pulse (metabolic problem). The average speed of the finishers was 15.2 km/h (range 13.2-17.8 km/h). Mean BM loss of the finishers near the ride midpoint was 4.2 ± 1.7%, as compared to 6.4 ± 2.9% in non-finishers (P = 0.04) but this difference was not apparent at the end of the race (5.7 ± 2.7% for finishers vs. 6.5 ± 3.1% at elimination, P = 0.58). Similarly, there were no significant differences in heart rate or veterinary assessment of hydration at the race end for finishers as compared to the elimination point for non-finishers. However, one of the horses eliminated for elevated pulse also had the greatest BM loss (11.5%) while the other had the most severe veterinary assessment of dehydration. Packed cell volume increased while sodium, chloride, and potassium concentrations decreased with exercise but no differences between finishers and non-finishers were observed. In contrast, ionized calcium concentration decreased in successful horses but remained unchanged in non-finishers.

Conclusion

Elite endurance horses are more likely to be eliminated from competition for lameness than metabolic problems; however, it remains unclear whether these conditions are entirely distinct. The difference in ionized calcium concentration between finishers and non-finishers is novel and warrants further study.

Gene expression profiling in blood cells of performer and disqualified endurance horses

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Introduction

By using cDNA microarrays, we proposed to show that the genes are modulated in leucocytes in relationship with the performance and clinical status of the horses. We assumed that disqualified horses for metabolic disorder may have some differential gene expression than performer horses. The aim of this study was to compare gene expression in leucocytes, haematological and biochemical parameters of performers versus disqualified endurance horses.

Material and methods

For biochemistry, haematology and genomic analysis, blood samples were collected at rest (T0) and just after a 140-160 km endurance race (T1) in two groups of horses: 10 performers (P) and 10 disqualified horses stopped for metabolic disorders (M). Total RNA was extracted from the blood cells (leucocytes), amplified and hybridised using mouse cDNA microarrays including 15264 unique genes. Differential gene expressions were studied by hybridisation of each sample T1 vs a control sample collected at T0 (pool of 20 sound horses).

Results

In group M, rhadomyolysis was confirmed with CK 13124 U/L and AST 1242 U/L. The list of 726 (including 603 annotated genes) significant genes was filtered according to a high p-value cut-off ($p<0.00001$). Among them, 130 were up-regulated (expression ratio>1.5) and 288 were down-regulated (<1/1.5). Sixty two genes were differentially expressed ($p<0.05$) in the two groups M and P. The expression levels of 28 and 50 genes were significantly correlated ($r>0.75$) with CK and AST level in group M, respectively. The gene ontology classification showed that more genes were up-regulated in the performer horses than in the disqualified horses. More genes were down-regulated in the disqualified horses.

Conclusion

Long exercise induced many significant gene modulations in leucocytes. Some genes are expressed in relationship with the rhabdomyolysis and haemolysis that were observed in the disqualified horses. Maybe some of these genes could be good gene candidates to explain some poor performance or pathologies.

The economic canter of endurance horses

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Introduction

The running economy in endurance horse should be a key point for performance. It was assumed that some canter styles are more efficient and more economic than others. The purpose of this study was to identify economic canter by measuring gait parameters of endurance horses of various ability.

Material and methods

The canter of thirty-three endurance horses were tested on a race track after 20 km of warming up exercise. They were 5 to 12 years old and performed according to endurance rules (40 km for young horses to 130 km for experimented horses). The rider was equipped with a GPS to keep the average speed of the canter at 18 km/h. Gait variables were measured by the accelerometric gait analysis system Equimetrix : stride frequency, dorsoventral displacement and power, angle and duration of propulsion and braking vectors. The total power was the sum of propulsion and braking during 10 s.

Results

A significant correlation between the power of the propulsion and its vector angle was observed: the greater the power of propulsion, the more horizontal the vector ($r = -0.7$). The braking showed the same correlation between angle and power ($r = 0.8$). Two groups of horses were observed according to the total power and angles ($P < 0.001$). One economical group was characterised by a lower total power (27.3 g ± 4), a lower braking angle (134 ± 92), a larger propulsive angle (49 ±0) and a good correlation between propulsive and braking power ($r = 0.7$). The other group was characterised by a great braking deceleration at the hoof impact and a great propulsion at the end of the diagonal stance phase. The results showed no effect of age or breed.

Conclusion

There were significantly different styles of canter. One style of canter was in the first group that had a more economical canter and was more adapted to the endurance race.

Trot asymmetry in relation to physical performance and metabolism in equine endurance rides

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Introduction

This study assessed if trot asymmetry appears along endurance competitions in successful and unsuccessful horses in relation to metabolism and lameness.

Material and methods

Fifty-eight horses were filmed at trot during the lameness examinations, before the competition (BF), and after phases 1 (29 Km), 2 (59 Km) and 3 (80 Km) and stride duration (SD) was measured in the four limbs. A locomotion symmetry index (LSI) was calculated by comparing SD in the diagonal left and right pair of limbs. Horses were considered symmetric when LSI was within X±2SD of the data obtained in the BF control. Venous samples were withdrawn immediately after the vet-gates, and WBC, PCV, creatinine, TPP, uric acid, AST, LDH, CK, Na, K and Cl were determined.

Results

The horses (68.97%) were successful, 17.24% were disqualified by lameness and 13.79% by metabolic diseases. Symmetric animals represented 70%, 75% and 90% of the sound, lame and metabolic groups respectively. In comparison to the asymmetric successful group, the symmetric horses presented lower mean velocity during the competition (12.18 vs. 14.83 Km/h) and plasma CK activity (408.4 vs. 846.3 IU/L), and higher velocity during the vet-gate controls (3.280 vs. 2.884 m/s), uric acid (72.40 vs. 40.26 mmol/L) and K concentrations (4.057 vs. 3.589 mmol/L). LSI became more asymmetrical along the competition in the sound successful group: 0.997, 0.996, 0.991 and 0.989 in the four controls. LSI was significantly different in the sound, lame and metabolic groups (0.993, 0.983 and 0.968 respectively). Significant correlations were observed between LSI, CK ($r = -0.990$), mean endurance velocity ($r = -0.978$) and mean velocity during the vet-gates ($r = -0.989$).

Conclusion

Although trot asymmetry increased progressively along the endurance events, it did not distinguish the sound animals from those eliminated due to lameness or metabolic problems. LSI seemed to be a useful index of muscle activity during endurance exercises.

Equine locomotor analysis on vet-gates in endurance events

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Introduction

This study was aimed at describing gait changes in sound horses along endurance events, since irregularities and gait alterations linked to fatigue might be difficult to distinguish from subtle lameness.

Material and methods

Seventy-five horses that completed an endurance event were filmed at trot during the previous examination (PCO) and at the vet-gates after phases 1 (PH1, 29 Km), 2 (PH2, 30 km) and at the end of 80 Km (PH3). Stride duration (SD), frequency (SF) and length (SL), and stance, swing, braking and propulsion durations expressed as a function of SD were measured in the four limbs.

Results

The horses trotted with a significant lower velocity during PH3 in comparison to PCO (2.309 ± 0.679 m/s, 3.365 ± 0.658 m/s). SF and SD were not different between the four controls, and a progressive reduction in SL was found (2.286 ± 0.386 m, 1.552 ± 0.452 m in PCO and PH3 respectively). Regression analysis confirmed that the horses did not change strategies between controls to obtain the same velocity. PH3 was characterized by increased stance (46.20 ± 4.705%, 38.49 ± 4.651%), braking (22.05 ± 2.539%, 16.96 ± 2.525%) and propulsion phases (24.15 ± 2.255%, 21.53 ± 2.417%) and decreased swing phase (53.80 ± 3.990%, 61.51 ± 4.171%) compared to PCO. In PCO, hind propulsion (23.03 ± 3.015%) and braking (18.79 ± 1.851%) were significantly longer than fore (21.53 ± 2.417%; 16.96 ± 2.524%). Conversely, in PH3, hindlimb propulsion was shorter (22.58 ± 2.955%) than in forelimbs (24.15 ± 2.955%). The fastest horses during the endurance competition used longer SL, and presented higher trotting velocity in the vet gates at PH1, PH2 and PH3 than the slowest.

Conclusion

Endurance horses trotted with a decreased velocity and SL in the vet-gate after 80 km, with this decrease being less evident in the fastest horses, although the strategies to reach the same velocity did not vary and the shorter hindlimb propulsion in PH3 revealed the development of physical fatigue.

Fatigue, stride parameters and hind limb stiffness in endurance horses

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Introduction

When a limb hits the ground, the muscles contract, stiffening the limb. With fatigue in human runners, limb stiffness (the ratio of force to change in leg length) decreases, reducing stability and increasing the chance for injury. The objective of the present study was to measure limb stiffness (using leg lengths) and stride parameters in non-fatigued and fatigued horses participating in an endurance ride.

Material and methods

Horses and riders were filmed (60 Hz) in the sagittal plane at mile 2 and mile 48 of an AERC-sanctioned 50-mile ride. An estimate of leg length was made from digitized (Image J) points on the dorsum of the hip and coronary band. Stride frequency (SF), time of contact (tc), and duty factor (DF) were determined. Data were analyzed using a paired t-test ($P < 0.05$). Useable video footage was obtained for 15 horses.

Results

Speed decreased from 4.6 to 4.0 m/s, but SF was not different (92 vs. 90 strides per minute). DF and tc were also unchanged. Hind leg length at mid stance was shorter at the finish (1.27 vs. 1.18 m) indicating a decrease in stiffness with fatigue because peak vertical force is independent of speed in the equine hind limb when trotting.

Conclusion

Even though fatigued horses were moving slower, SF, DF, and tc were not different-a pattern observed in some studies in human fatigue. It remains to be determined if a decrease in limb stiffness, coupled with the stride parameters are adaptive and aid in reducing injury.

Time-induced changes in hematological and biochemical parameters over a 140 km endurance race in a group of national level horses

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Introduction

The aim of this field study was to identify interesting trends in blood parameters used to assess fatigue in top endurance horses over the 36 hours preceding and following a race.

Materials and methods

31 top endurance horses were sampled the day before the race (T0), at the end of a 140 km long endurance race (T1) and the morning after (T2). Complete blood counts were obtained, and serum/plasma total proteins, fibrinogen, CK, SGOT, LDH, electrolytes, total bilirubin, bile acids, f×GT, urea, creatinine, lactate, glucose and triglycerides were measured. An analysis of variance was performed on pooled results of all horses at T0, T1 and T2.

Results

13 horses finished the race and 18 horses were eliminated. Amongst the eliminated horses, T1 samples were pulled at the time of elimination. (7/18 raced over a distance > 110 km and 14 /18 raced over a distance > 90 km).Red blood cell parameters (Hematocrit; Hemoglobin; RBC count) increased significantly (+ 23-25%) with exercise ($P < 0.05$) and returned to the baseline within 24 hours. Conversely, a stress-induced neutrophilia (+ 28%; $P < 0.05$) subsided within 24 hours after the race. All muscle enzymes increased with exercise, but only CK can be used statistically over the period studied (+ 2200%; $P < 0.05$). Serum protein decreased (-12%) after the race ($P < 0.05$) while urea increased (+ 28%) with exercise and elevation persisted at least 12h after the race ($P < 0.05$). Serum creatinine increased (+ 40%; $P < 0.05$) with the race and returned to the baseline the next day. Plasma fibrinogen increased with the race (+ 13%; $P < 0.05$) but this was significant only the day after. Liver function did not appear to be significantly affected by exercise. Variations in serum electrolytes were noted, but only changes in chloremia, kalemia and magnesemia reached statistical significance ($P < 0.05$).

Conclusion

Stress and fatigue induced by a long-distance race are clearly expressed by changes in red and white blood cells as well as creatinine. Tissue remodelling is highlighted by changes in fibrinogen, urea, proteins and CK. The data on energetic metabolism were too incomplete to interpret over time.

Measuring core body temperature of endurance horses

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Introduction

Endurance horse competitions are now conducted throughout the world, including hotter, more humid climates, where it is challenging for the horses to dissipate the heat produced during exercise, especially at the increasing pace of modern endurance rides. Although rectal temperatures may be taken at rest stops, there is little information about core body temperatures during the exercise, and there has been no continual monitoring of horses during rides. The use of internally placed temperature loggers, which can record for several weeks, will allow the collection of data from several rides, under differing environmental conditions.

Material and methods

Calibrated and coated i-buttons (Dallas Semiconductor, TX, USA) were placed transcervically into the uteri of endurance mares, and left in utero for 4 weeks, while the horses trained and competed as normal in endurance competitions. External environmental loggers (HOBO, Onset Computer, MA, USA) were fitted to the horses for the competitions. Data from the devices were downloaded, to determine the effect of endurance competition on core body temperature.

Results

Preliminary results show that the internal loggers reliably recorded the temperatures of the mares. Further work is proceeding to describe the effect of endurance competition on temperatures, and determine any relationship with external environmental conditions.

Conclusion

We have established that this non-surgical technique reliably records the core body temperature of horses over several weeks, and will now be used to test the effects of different environments, levels of exertion, and methods of cooling post exercise on core temperature of endurance horses.

Uric acid responses to endurance racing and relationships with performance, plasma biochemistry and metabolic alterations

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Introduction

The study was undertaken in order to better understand the uric acid response to endurance races.

Material and methods

Blood samples were taken from horses the day before and 5-10 minutes after successfully finishing a 120 km (assay 1, n = 24) or a 160 km endurance race (assay 2, n = 17), and from 19 animals eliminated by metabolic disorders during several endurance races (assay 3). Plasma was obtained and determination of CPK, AST, LDH, AP, uric acid (UA), creatinine, urea, lactate, glucose and plasma proteins (PP) were carried out. Sex, age, time in competition, average speed and total recovery time were also recorded. Assay 1 and 2 were arithmetically subdivided in 3 groups in order to categorize time in competition, average speed and total recovery time.

Results

Average speed was higher in assay 1 compared to assay 2. However, there were no differences in plasma biochemistry values between these two groups. The fastest horses (assays 1 and 2) showed higher UA levels, while the slowest horses (assay 2) showed less UA levels. The animals with metabolic alterations had higher UA, CPK and PP compared with those that adequately concluded the race. There were significant correlations between UA and CPK in assay 1 ($r = 0.46$), assay 2 ($r = 0.41$) and assay 3 ($r = 0.51$), and between UA and PP in assay 1 ($r = 0.39$) and assay 3 ($r = 0.47$).

Conclusion

UA rises in horses after a prolonged effort, with this increase higher in animals with metabolic commitment and in the fastest horses. This increase has a direct correlation with CPK.

Is potassium supplementation beneficial during intense endurance exercise?

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Introduction

Increases in plasma [K+] occur with exercise and supplementation of potassium (K) may further increase plasma levels, increasing the risk of neuromuscular hyperexcitability. Higher K content increases dietary cation anion difference (DCAD), which would tend to decrease plasma [Ca++], exacerbating hyperexcitability. This study compared the effects of a K-rich electrolyte supplement (EM+K) to a K-free one (EM-K) on plasma [K+], [Ca++] and acid-base status during a treadmill exercise test in horses.

Material and methods

The treadmill test consisted of three bouts (simulating loops in an endurance race) of 12 km (at 6, 7, and 8 m/s) with two intermediate 25 minute rests (R) on thirteen endurance Arabian horses. Electrolytes were supplied orally 60 min before exercise and at each R. Blood samples were taken before (PRE), during exercise, R1, R2 and 150 min of recovery (REC). Blood was analyzed for pH, PCO2, plasma ionized electrolytes, glucose, and lactate; plasma [H+] was calculated. The DCAD was calculated to be -27 mEq EM-K and 109 mEq in EM+K, per dose.

Results

Plasma [H+] decreased during the first two loops, increased during the 8 m/s loop, and returned to PRE at R and REC ($P < 0.020$). Plasma [K+] was higher at 8 m/s ($P < 0.050$) and plasma [Ca++] was overall lower ($P = 0.020$) in the EM+K group compared to EM-K. The use of EM-K avoided increased plasma [K+] at higher speeds. Acute effects of a lower DCAD in EM-K may have led to higher plasma [Ca++].

Conclusion

Lower plasma [K+] and higher plasma [Ca++] with EM-K supplementation may help prevent signs of neuromuscular hyperexcitability and avoid associated clinical problems during higher speeds in endurance races.

Serum levels of cardiac troponin I in horses after prolonged endurance exercise

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Introduction

Cardiac troponin I (cTnI) is the biomarker with the best sensibility and specificity to myocardial injury. With the purpose of evaluating whether long-term exercise and metabolic compromises would lead to myocardial injuries, serum cTnI was evaluated before and after endurance competitions.

Material and methods

Blood samples were collected from horses submitted to endurance races with distances from 42 to 160 Km, divided into the following : Control: 33 samples collected before the competitions; G1: 24 horses which completed the races; G2: 14 horses disqualified due to metabolic causes; and G3: 15 horses disqualified due to lameness, all samples in a maximal 60 min post-exercise interval. Cardiac TnI levels were determined with a chemiluminescent immunometric assay. Values of > 0.5 ng/mL were considered positive.

Results

The median (Q1-Q3) values for serum cTnI, in ng/mL, were 0.200 (0.125-0.290) for Control, 0.205 (0.155-0.487) for G1, 0.095 (0.047-0,177) for G2, and 0.180 (0.120-0.230) for G3. The proportion of positive cTnI individuals for groups C, G1, G2 and G3 were, respectively, 9.09%, 25.00%, 0.00% and 6.67%.

Conclusion

Although there was only a significant difference between G2 (lower values, $P < 0.04$) and the other groups, long-term strenuous exercise seemed to lead to minor myocardial lesions, due to a higher cardiac workload, since horses which completed the endurance races (covering significantly greater distances) tended to present slightly higher values and a greater proportion of positive values. The metabolic causes of disqualification during the competitions appeared not to be related to myocardial injury and the horses disqualified due to lameness had a tendency to have a greater cardiac workload than the horses disqualified due to metabolic causes.

Analysis of peripheral blood mononuclear cell gene expression in endurance horses by the cDNA-AFLP technique

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Introduction

The knowledge of molecular mechanisms of stress response in athlete horses can allow us to plan an appropriate and high-grade training. It is well known that excessive muscular exercise can lead to a number of responses influencing hypothalamic-pituitary-adrenocortical axis and sympathetic system functions which may be associated with modification of the mRNA levels for a number of metabolic genes such as those involved in the immune response.

Material and methods

In the present study, the cDNA-AFLP technique was applied to Arab endurance horse PBMC under stressing conditions to visualise variations of transcriptional profile.

Results

Forty-nine transcript derived fragments (TDF), differentially expressed, were cloned and sequenced. Four of these showed high sequence similarity with genes probably involved in exercise-induced stress response and resulted to be not sequenced in the horse. Their modulation was confirmed by RT-PCR and the full-length transcripts were isolated by RACE-PCR. The mRNA sequences obtained were included in the GenBank database as *Equus caballus* interleukin 8 (IL8), *Equus caballus* retinoblastoma binding protein 6 mRNA (RBBP6), *Equus caballus* eukaryotic translation initiation factor 4 gamma 3 (eIF4G3) and *Equus caballus* heat shock protein 90 (Hsp90).
The expression pattern of these genes was verified in other endurance horses under stressing conditions, strengthening the hypothesis of their real involvement in exercise stress-induced response.

Conclusion

The cDNA-AFLP technique used to evaluate physical stress in endurance horses allows the modification in gene expression to be determined. This can be used for the evaluation of the over training syndrome. In addition, it can help to preserve animal welfare and to formulate appropriate training plans.