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RESEARCH ARTICLE
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Serum muscle-derived enzymes response during show jumping competition in horse

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Abstract

Aim: The effect of two jumping competitions, performed in two consecutive weekends, on serum creatine phosphokinase (CPK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH), urea, creatinine (CREA) concentrations were evaluated in 12 healthy jumper horses.

Materials and Methods: Blood sampling was performed before the 1st day of competition (T_0), at the end of each show (J_1, J_2), on the day after the competition (T_1); the same sampling plan was followed during the second weekend (J_3, J_4 and T_2).

Results: One-way repeated measures analysis of variance showed an increase in CPK at J_1 and J_2 respect to T_0 and at J_3 and J_4 respect to all other time points ($p<0.05$). LDH activity showed an increase at J_2 respect to T_0, at J_3 respect to T_0, J_1, J_2 and at J_4 respect to all other time points ($p<0.05$). AST values increased at J_1 and J_2 respect to T_0 ($p<0.05$). A significant increase of CREA was found at J_3 respect to T_0, T_1 and J_1 and at J_4 respect to all other time points ($p<0.05$). A decrease in serum urea levels was found at J_1 respect to T_0, at J_2 and J_4 respect to T_0 and T_1; at T_2 respect to T_0 ($p<0.05$). A positive correlation between urea/CPK ($p=0.0042$, $r^2=0.030$), LDH/CPK ($p<0.0001$, $r^2=0.535$), CREA/LDH ($p<0.0001$, $r^2=0.263$), CREA/CPK ($p<0.0001$, $r^2=0.496$) was observed.

Conclusion: Our results suggest that 5 days recovery period between the two consecutive competition weekends is insufficient to allow muscle recovery and avoid potential additional stress. The findings obtained in this study improve the knowledge about metabolic changes occurring in athlete horse during the competition to identify muscle alterations following show jumping competitions.

Keywords: horse, muscle enzymes, physical exercise, show jumping competition.

Introduction

The performance of athletic horse is determined by many complicated interdependent biological and physiological processes. Similarity to other stressors, including delivery, transport and environmental conditions exercise need adequate response to re-establish homeostatic equilibrium [1]. Several cardiovascular and hematological adaptations are necessary to guarantee the correct supply of oxygen to active muscles during exercise. Physiological, hematological, and biochemical changes associated with exercise have been extensively analyzed in several types of horses such as thoroughbreds [2,3] eventers [4,5], show jumpers [6-9], and endurance horses [10,11].

One of the organs affected by exercise is the muscle, which suffers microdamage due to effort employed load [12]. Many researchers have compared muscular adaptations that occur after several training programs with different exercise intensities [13,14], others have examined the combined effect of intensity and duration of the exercise [15], or assessed adequate recovery after exercise.

Repeated workload leads the muscular apparatus to constant damage that can be assessed by laboratorial determination of some serum constituent such as urea and creatinine (CREA) and enzymes such as creatine phosphokinase (CPK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) [16]. The activity of these enzymes has been studied by several researchers before and after exercise and can be used to detect muscle diseases [17], characterization of exercise intensity [11,18] and predicting possible complications that can arise from the exercise [19]. Despite the existing literature describing muscular adaptations to training in horses, little is still known about the durations and intensities of exercise that promote optimal response

in skeletal muscles [20]. Considering the metabolic and clinical role of serum parameters above mentioned and considering the effect of physical exercise on them, studying changes in these muscle damage markers after exercise has become more important [21].

Therefore, the objective of this study was to evaluate the serum concentration of urea, CREA and the serum activities of CPK, AST and LDH of jumper horses before and after jumping competition.

Materials and Methods

Ethical approval

Protocols of animal husbandry and experimentation were reviewed and approved in accordance with the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU for animal experiments.

Animals

The study was carried out on 12 healthy and regularly trained Italian Saddle horses (7 geldings and 5 females, 9-12-year-old, mean body weight 500±20 kg). All horses were managed equally, housed in individual boxes under natural photoperiod (mean temperature 25±6°C, relative humidity 67±3%). The horses were fed standard rations, calculated to fulfill all the nutritional requirements according to National Institute of Agronomic Research specifications [22] constituted hay (first cut meadow hay, sun cured, late cut, and a mixture of cereals) oats and barley, 50% each.

The percentage composition of the mixture was dry matter 87% and moisture 13%. The dry matter contained 9.11% digestible protein, 13.05% crude protein, 20.7% crude fiber, and 3.42% crude lipid, as well as 0.80 Unitè Fouragire Cheval/kg. The ration was administered 3 times a day: 8:00 AM, 12:00 PM and 5:00 PM. Water was available *ad libitum*.

Show jumping course

Horses took part in jumping competitions held in two consecutive courses at distance of 5 days. Each completion session was preceded by 20 min warm-up consisting of walk, trot and gallop with six jumps (height: From 100 to 140 cm). During the 1st day of both weekends, horses competed with the following technical specifications: Total length - 550 m; obstacles height - 140 cm; total efforts 13 (7 verticals, 6 oxers, 1 triple combination). During the 2nd day of both weekend competitions, horses competed with the following technical specifications: Total length - 600 m; obstacles height - 145 cm; and mixed competition including efforts 15 (8 verticals, 7 oxers, 1 double combination, 1 triple combination).

All horses during the week between both competitions performed the following daily training schedule warm-up (10 min walk, 20 min trot, 10 min gallop) and show jumping course with 7 fences of 80±10 cm average height.

Blood sampling and analysis

Blood samples were collected by jugular venipuncture in vacutainer tubes with cloth activator for serum analyses (Terumo Co., Tokyo, Japan). Blood sampling was performed before the 1st day of competition (T_0), within 10 min from the end of each competition (J_1, J_2) and on the day after competition (T_1), same plan was followed during second weekend (J_3, J_4, and T_2). Immediately after collection, blood samples were placed in refrigerated bags and transported to the laboratory for the analysis. Tubes were centrifuged at 3000 rpm for 10 min and on obtained sera the concentration of CPK, LDH, AST, urea, CREA were determined by commercial available kit by means of an automated analyzer, Model 7070 (Hitachi Ltd., Tokyo).

The same operator assayed all samples in duplicate each time. Samples exhibited parallel displacement to the standard curve; the intra-assay and the inter-assay coefficients of variation were <7% and <9%, respectively, for all measured parameters.

Statistical analysis

The obtained data are expressed as mean ± standard deviation (SD) of the mean. Data were normally distributed ($p>0.05$, Kolmogorov–Smirnov test). One-way repeated measures analysis of variance (ANOVA) was applied to determine the statistically significant effect of exercise on all parameters. $p<0.05$ were considered statistically significant. Bonferroni's multiple comparison tests were applied for *post-hoc* comparison. A simple linear regression model was applied to evaluate the correlation between the studied parameters. A statistical analysis was performed using Stats package of R Core Team (2013) (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, 2013, URL: http://www.R-project.org/).

Results

ANOVA showed an increase in CPK at J_1 and J_2 respect to T_0 and at J_3 and J_4 respect to all other time points. LDH activity showed an increase at J_2 respect to T_0, at J_3 respect to T_0, J_1, J_2 and at J_4 respect to all other time points. AST values increased at J_1 and J_2 respect to T_0. A significant increase of CREA was found at J_3 respect to T_0, T_1 and J_1 and at J_4 respect to all other time points. A decrease in serum urea levels was found at J_1 respect to T_0, at J_2 and J_4 respect to T_0 and T_1; at T_2 respect to T_0.

A positive correlation between CREA/LDH ($p<0.0001$, $r^2=0.263$), CREA/CPK ($p<0.0001$, $r^2=0.496$) urea/CPK ($p=0.0042$, $r^2=0.030$), LDH/CPK ($p<0.0001$, $r^2=0.535$), was observed (Figure-1).

Discussion

The obtained results showed that hematochemical modifications occur after exercise in jumper horses. With regard to the muscle enzymes, an increase

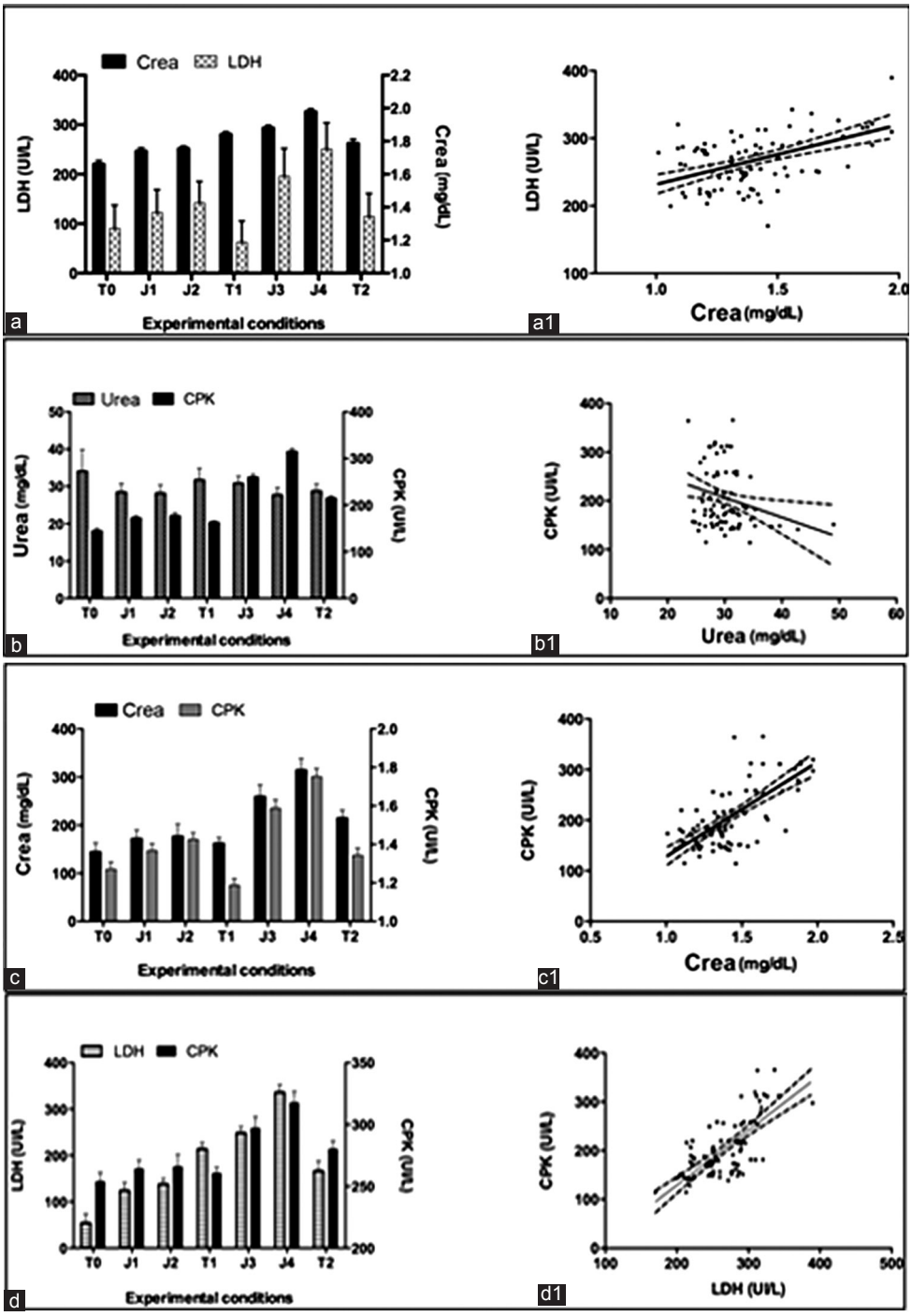

Figure-1: (a) Pattern of serum creatinine (CREA) and lactic dehydrogenase (LDH) observed during experimental period; (a1) Graphical representation of simple linear regression of LDH vs. CREA; (b) Pattern of CREA and creatine phosphokinase (CPK) observed during experimental period; (b1) Graphical representation of simple linear regression of CPK versus CREA, (c) Pattern of serum urea and CPK observed during experimental period; (c1) Graphical representation of simple linear regression of CPK versus urea; (d) Pattern of LDH and CPK observed during experimental period; (d1) graphical representation of simple linear regression of CPK versus LDH.

in the post-exercise activities of CPK, AST and LDH was fond compared to baseline values measured at T_0. According to our results, increase in serum CPK, AST, and LDH activities have been seen in response to exercise [23]. These increases are believed to relate either to overt damage or to a change in the muscle fiber membrane causing a transient increase in permeability [24]. However, physiological increases have been also shown to occur without any tissue alteration [25]. The effects of physical effort on serum enzymatic activity may depend on the level of performance of the animal, and the intensity and duration of exercise [26].

A significant increase of CPK was observed after the competitions. The post-exercise increase of CPK levels could be attributed to the muscle metabolism and to the increasing energy requirements occurring during physical exercise [27]. It is well stated

that the tissue CPK activity may augment energetic capacity and improve myofibril contraction responses through enhancing vascular tone and vasoconstrictor reserves [27]. At T_1, it showed slightly, but no significant decrease respect to J_1 and J_2, although remained highest, but no significantly, respect to T_0. This probably because blood sampling at T_1 occurred 24 h after the first weekend of competition and this is an insufficient time to determine a return to normal serum concentration. During the second weekend of competition, CPK assumed higher levels respect to the previous week and reached its peak levels in J_4. Its values remained significantly higher at T_2 respect to T_0 assuming that the shorter recovery period to the first competition (only 4 days) associated with another rise post-exercise, could increase the probability of muscle damage. CPK has relatively shorter half-life of 2 h [28]. Therefore, will become elevated sooner and return to normal range after an episode of muscle strain, unless the effort is so high as to delay its return to the baseline levels. For this reason, it is preferred to evaluate muscle enzyme for diagnosing and monitoring muscle recovery and considered a reliable marker of skeletal muscle injury [28]. LDH activity showed a significant increase at J_2 respect to T_0, probably due both to the increased effort respect to J_1 and to the increase of enzyme activity after 24 h post exercise (T_1). LDH have a longer half-life respect to CPK, in fact, it peaks 24 h after effort and could remain high for 48 h after exercise [11]. Therefore, since blood sampling at T_1 occurred 24 h after the last jumping round of the 1st week of completion, return to baseline levels could not be determined. Our study showed a greater increase in the levels of this enzyme at J_3 and J_4 revealing, as well as in CPK activity, a reduced capacity to recovery by effort probably due to the horses have performed competitions in two consecutive weekend.

AST activities may increase during exercise without observation of clinical signs or histological detection of changes in muscle cell structure [29]. In our study, AST values increased immediately after exercise, whereas they decreased at T_1 reaching levels measured at T_0.

In this study, a slightly but significant increase of CREA was found in J_3 and J_4. CREA is produced from the decomposition of creatine, a nitrogen compound used by muscle cells to store energy. The serum concentration of CREA varies according to creatine synthesis and the amount of muscle mass and exercise as reported by Nogueira *et al*. [30] in thoroughbred.

During the exercise phases and the recovery period, all animals showed a decrease in serum Urea levels respect to T_0. Urea is filtered by the glomerular capillaries, and it enters the renal tubule. Approximately half of urea is reabsorbed passively by diffusion, but the remainder is excreted in the urine. Lower urea levels suggest an increased glomerular filtration and an excretion in the urine or a diminished reabsorption in the tubules.

On the basis of our results, we can affirm that the higher levels of CPK and LDH in J_2 and J_4 (increased effort) respect to J_1 and J_3 confirmed that the effects of physical effort on serum activities of muscle enzymes is strictly linked with intensity and duration of exercise; the increased levels of CPK and LDH occurred during the second weekend of competition (J_3 and J_4) respect to the first, seem to indicate that the two jumping session were temporally too close and did not allow the horse adequate recovery.

Conclusion

Our results improved the knowledge about metabolic changes occurring in athlete horse during competition and underline that the physiological activity of enzymes, if not associated with the adequate recovery period, can increase the probability of muscle damage.

Authors' Contributions

AA and GP designed the study and supervised the research as major advisor. SM, FC and FF worked and collaborated in the lab work and compilation of the results as well as the manuscript. GC and DB provided valuable suggestions regarding the design of the experiment and analysis of the data collected during research. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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