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Blood biochemical changes in show jumpers during a simulated show jumping test

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ABSTRACT

The modifications of some biochemical parameters and serum electrolytes after a simulated show jumping test were evaluated. On seven clinically healthy Italian saddle horses, heart rate (HR), lactate concentration, serum concentrations of alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), creatine kinase (CK), gamma glutamil transferase (γGT), lactic dehidrogenase (LDH), creatinine, urea, total bilirubin, glucose, chloride (Cl^-), potassium (K^+) and sodium (Na^+) were analysed at rest, after the warm up, immediately after the simulated show jumping and 45 minutes after the end of the exercise. One-way repeated measures analysis of variance (ANOVA) showed a significant effect of sampling time ($P<0.05$) on HR, lactate concentration, ALP, AST, ALT, CK, γGT, creatinine, glucose and K^+. These results suggest that by knowing the biochemical changes during a simulated jumping test, the veterinarian could better judge the horses with electrolytes and metabolic disturbances in a competition.

Key words: electrolytes, biochemical parameters, horse, show jumping test

Introduction

Show jumping, the main equestrian sport in Italy, has an energetic requirement strictly dependent on the length of the course and on the height of fences, and it requires several years of training to develop the full potential of a horse (CLAYTON, 1991). The training of jumpers involves the utilisation of periodic exercises that cause structural, functional and behavioural changes, preparing them for the competitions (FERRAZ et al., 2010). An effective response to training depends on the use of appropriate stimuli which should be guided by the principle of overwork, made up of three classic variables represented

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by the triad of: intensity, duration and frequency (EVANS, 2000). The performance of the athlete is determined by many complicated interdependent haematochemical and physiological processes (WARWICK, 2004). In fact, competition regulations require that veterinary judges protect the health and welfare of the horses, based particularly on the measurement of heart rate and some variables utilised for evaluation of workload effort, such as lactate concentration (ROGERS et al., 2007; TRIGO et al., 2010). According to LINDINGER and HEINGENHAUSER (2008), other parameters can be used to determine the effect of exercise, such as glucose, enzymatic and haematochemical parameters, and electrolytes (Na^+, K^+ and Cl^-) with the purpose of defining reliable parameters for the horse's performance assessment. In the horse, electrolytes play an important role in maintaining osmotic pressure, fluid balance, and nerve and muscle activity (FRAPE, 2010). So, it is important to have some idea of the magnitude of loss of electrolytes from a horse during exercise (VAN DEN BERG, 2009).

Since it is important to analyse the modifications of these parameters in the function of different systems and types of energy utilized (DE MIRANDA et al., 2009), haematological, biochemical and electrolytic parameters have largely been evaluated during different kinds of physical effort, such as trot races (TATEO et al., 2008; PICCIONE et al., 2008a; PICCIONE et al., 2009) and endurance training and racing (LINDINGER and HEINGENHAUSER, 2008; PICCIONE et al., 2008b; ROBERT et al., 2010; MUÑOZ et al., 2010). In jumpers many studies have been carried out with the purpose of defining the pattern of some physiological, haematochemical and haematological changes during training and physical exercise (LEKEUX et al., 1991; BARREY and VALETTE, 1993) showing significant variations of some of these parameters after training or event. Since show jumping represents an intense muscular exertion which requires some anaerobic metabolism, physiological and metabolic responses in athlete horses participating in show jumping competitions have been presented (PICCIONE et al., 2007). So, the aim of this study was to evaluate the feasibility of biochemical and electrolytic analysis in show jumping.

Materials and methods

The study involved a laboratory component and a veterinary clinic component, both conducted at the University of Messina's School of Veterinary Medicine. 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 by Directive 86/609 CEE. The study was carried out in the spring (April 2010) in Sicily on seven Italian saddle horses (4 geldings and 3 female) with a mean age of 9 ± 3 years and mean body weight of 480 ± 70 kg. All horses were weighed using a horse electronic balance (Sm Trade & Technology S.R.L., Repubblica di San Marino). Before the start of the study, all subjects underwent a laboratory and clinical examination in

order to exclude animals with injuries, swellings or any form of apparent disease (Table 1). General animal care was performed by professional staff not associated with the research team. All horses, clinically healthy, were traditionally trained for a period of 60 days, six days a week with one day of rest on Sundays. All horses were trained with the same traditional training protocol and achieved the same level of performance and fitness. Training lasted about 1 hour each day and included walking, trotting, galloping and obstacle jumping. After the training period, all horses were subjected to the testing protocol shown in Fig. 1.

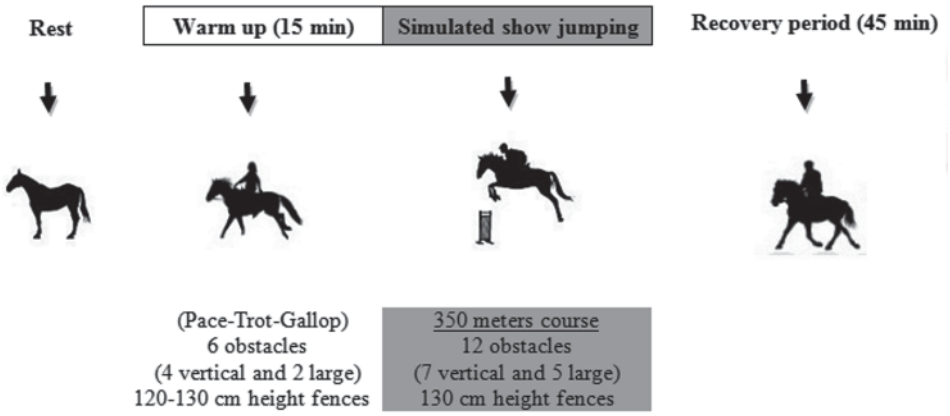

Fig. 1. Testing protocol applied to seven jumper horses.

Training and general animal care were performed by professional staff not associated with the research team. All horses were kept in individual boxes under a natural photoperiod and natural indoor temperature (18-20 °C). The horses were fed a standard ration, in amounts calculated according to the natural requirements assessed by INRA (Institut National de la Richerche Agronomique) specifications (MARTIN ROSSET, 1990), at 8:00 AM, 12:00 AM and 5:00 PM each day. The ration, without supplementation of electrolytes, consisted of hay (first cut meadow hay, sun cured, late cut, 8 kg/horse/day, 6.9% crude protein on average) and a mixture of cereals (oats and barley, 50% each, about 3.5 kg/horse/day). The composition of the mixture of cereals was as follows: dry matter 87%; moisture 13%; horse digestible protein (MADC) 9.1% on dry matter, crude protein 12.1% on dry matter, crude fibre 20.7% on dry matter, ether extract 3.4% on dry matter, UFC/kg dry matter 0.80. Water was available *ad libitum*. The time between jumping test and feeding was 2 hours. In order to evaluate the workload during the experimental period, for each horse the heart rate (HR) was measured with a Polar® S610i™ sensor

(Polar Electro Oy, Kempele, Finland) at rest, after the warm up, immediately after the simulated show jumping test and 45 min after the end of the show jumping (recovery period characterized by 10 minutes' walking). The blood for immediate assessment of blood lactate concentration was collected by jugular venipuncture and analyzed with a portable blood lactate analyser (Accusport Bohering, Germany). Moreover, from all subjects blood samples were collected by jugular venipuncture using vacutainer tubes (Terumo Corporation, Japan) with no additive at 4 different sampling times: at rest, after the warm up, immediately after exercise, and 45 min after the end of exercise. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes and the obtained sera were separated and stored at -20 °C until analysis. From the obtained sera, concentrations of alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), creatine kinase (CK), gamma glutamil transferase (γGT), lactic dehidrogenase (LDH), creatinine, urea, total bilirubin, glucose, chloride (Cl^-), potassium (K^+) and sodium (Na^+) were analyzed with commercially available kits by means of a UV spectrophotometer (model Slim SEAC, Florence, Italy). One-way repeated measures analysis of variance (ANOVA) was applied to evaluate the effect of sampling time on blood biochemistry parameters. Newman-Keuls's test was applied for post hoc comparison. A P value<0.05 was considered statistically significant. All data were analysed using the Statistica 7.5 software package (Stat Soft Inc., USA).

Results

The duration of the simulated show jumping test was 70 seconds.

All the results presented in Tables 1-2 are the obtained average values together with the relative statistical significances of the studied parameters, expressed in their unit of measurement. Statistical analysis showed a significant increase after the warm up and the simulated show jumping test, and consequently a decrease down to baseline during the recovery period for the following parameters: HR ($P<0.0001$; $F_{(3;27)} = 294.00$), lactate concentration ($P<0.0001$; $F_{(3;27)} = 61.45$), ALP ($P<0.001$; $F_{(3;27)} = 131.80$), AST ($P<0.0001$; $F_{(3;27)} = 21.12$), ALT ($P<0.0001$; $F_{(3;27)} = 11.47$), CK ($P<0.001$; $F_{(3;27)} = 58.07$), γGT ($P<0.0001$; $F_{(3;27)} = 68.88$), creatinine ($P<0.0001$; $F_{(3;27)} = 144.20$), glucose ($P<0.001$; $F_{(3;27)} = 2.57$) and K^+ ($P<0.0001$; $F_{(3;27)} = 18.50$).

Table 1. Comparison of haematological and clotting parameters (mean values and standard deviations), expressed in their conventional units of measurement, of seven healthy Italian saddle horses, with reference ranges

Parameters	Units of measurement	Mean values	Standard deviations	Ranges (THRALL et al., 2004)
RBC	M/µL	7.22	0.89	6.5 - 13
WBC	K/ µL	6.32	1.14	5 - 12
HGB	g/dL	11.40	1.44	12 - 19
PCV	%	34.36	8.35	32 - 53
MCV	fL	46.65	3.02	34 - 50
MCH	pg	15.81	1.16	13 - 20
MCHC	g/dL	33.89	0.99	32 - 39
PLT	K/ µL	138.10	50.33	120 - 400
MPV	fL	8.27	1.16	6 - 11
Rectal temperature	°C	37.8	0.7	37.2 - 38.6
Respiratory rate	breaths/min	14	2	12 - 16

Table 2. Mean values (±SD) of blood lactate and heart rate, expressed in their units of measurement with the statistical significances, in seven Italian saddle horses, during the testing protocol

Parameters		Testing protocol		
	At rest	After the warm up	Simulated show jumping test	Recovery period (45 min)
Blood lactate (mmol/L)	1.41 ± 0.33	2.24 ± 0.67*	5.00 ± 0.46$^{*\blacklozenge}$	2.52 ± 0.70$^{*\circ}$
Heart rate (bpm)	38 ± 7.07	120 ± 14.14*	200 ± 23.80$^{*\blacklozenge}$	48 ± 6.95$^{\blacklozenge\circ}$

* *vs* At rest P<0.05; $^{\blacklozenge}$ *vs* After the warm up P<0.001; $^{\circ}$ *vs* Simulated show jumping test P<0.001

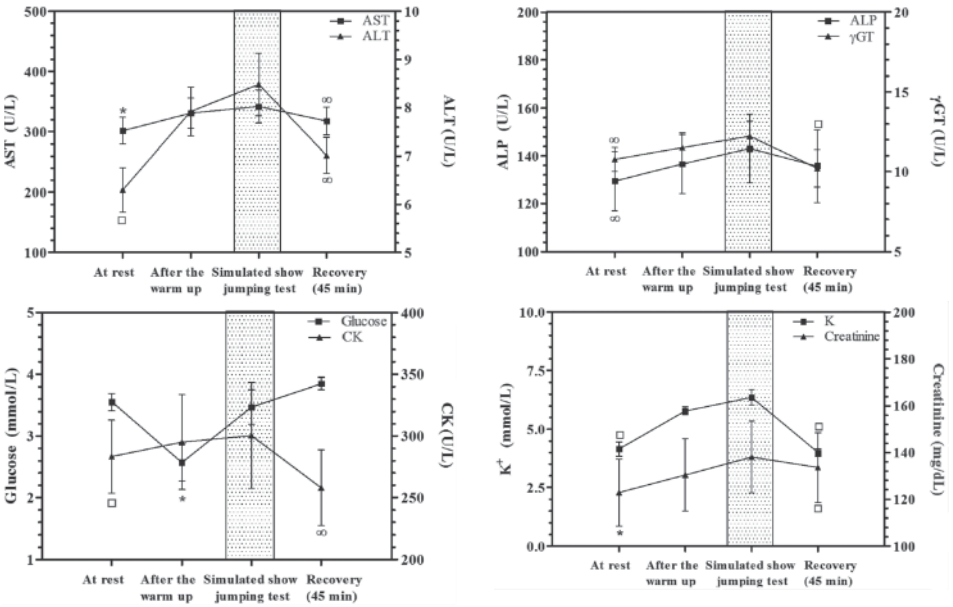

Fig. 2. The pattern of mean value (± SEM) of AST (U/L), ALT (U/L), ALP (U/L) γGT (U/L), glucose (mmol/L), CK (U/L), Creatinine (mg/dL) and K^+ (mmol/L), obtained in 7 jumper horses during the testing protocol. * - vs all times of testing protocol (P<0.01); □ - vs after warm up and simulated show jumping test (P<0.01); ∝ - vs simulated show jumping test (P<0.01).

Discussion

The results of this study showed the statistically significant effect of the simulated show jumping test on some of the parameters studied. Particularly, we quantified the workload of the testing protocol by monitoring heart rate and blood lactate concentrations, which are traditional parameters in the evaluation of horse performance (CLAYTON, 1994). The results shown in Table 2 indicate that adequate levels of exertion were attained in our study, in which exercise involved anaerobic and aerobic metabolism, and the recovery time (45 minutes) was in accordance with the values reported in the previously published data (FAZIO et al., 2010). Moreover, our findings show that the show jumping test significantly influences some studied parameters. For example, the glucose values showed a significant decrease after the warm up and a significant increase after the show jumping test and 45 min after the end of the exercise, with similar values as baseline values observed at rest. The possible explanation of this decrease would be the greater muscle uptake of blood glucose, which is consistent with activation of the glycolytic pathways, while the

increase in glucose in the test could be due to haemo-concentration or activation of the fat metabolism. The purpose of the warm up, in fact, is to adjust the body to transition from rest to exercise, and during this phase a gradual increase in the supply of energy fuel to the muscle is released, so glycogenolysis and lipolysis are activated from the catecholamines (HINCHCLIFF et al., 2008). Also, all parameters involved with functions of the liver, such as ALP, AST and ALT, displayed a significant increase after the show jumping test. As is well known, the activities of various enzymes increase following muscle damage or strenuous muscular exercise (ANDERSON, 1975; KEER and SNOW, 1983; BALOGH et al., 2001). The generally modest increases, found in muscle-derived enzymes in the plasma or serum in response to both low- and high- intensity exercise, have been suggested to reflect increases in mitochondrial membrane permeability, rather than muscle damage (HODGSON and ROSE, 1994; VALBERG, 2009). The increase recorded after the show jumping test and the decrease during the recovery period for γGT indicated the increased work of the liver, as previously demonstrated in humans in whom it has long been known that physical exercise results in the transient elevation of liver function (PETTERSSON et al., 2007). The parameters directly related to the muscular work, such as CK, also showed a similar trend. As shown by our results, these parameters reached a peak after the show jumping event, but already showed an increase during the warm up period. The increase in CK reflects an increase in mitochondrial membrane permeability, rather than muscle damage (HODGSONE and ROSE, 1994). Moreover, in our study the electrolytes did not show any significant variation except for K^+. In particular, a significantly increased value was recorded for this parameter during the warm up and after the show jumping test, whereas a significant decrease was observed during the recovery period. K^+, the major intracellular cation, is important for osmosis and normal balance of water and cellular biochemical functions (SANTOS et al., 2001). Its metabolism is very closely regulated by the hormones of the kidneys, adrenal glands thyroid and pancreas, usually this electrolyte is almost entirely absorbed from the small intestine, and it is excreted by the kidneys, sweat, faeces and skin cells (HESS et al., 2005). The increase in the serum potassium levels after exercise, as already suggested by a previous study, may be due to the changes to muscle fibre content after recruitment (PICCIONE et al., 2007); in fact immediately after exercise potassium is taken up again by the muscle cells that are under $beta_2$ adrenergic receptor control.

Conclusion

If a careful interpretation of the data reported in our study is made, our findings will be important to assist veterinarians in their judgement during show jumping competitions. The results of this study suggest that biochemical data, alone or combined, could help to identify horses with an increased risk of developing alterations before their elimination during competitive show jumping events.

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FAZIO, F., S. CASELLA, A. ASSENZA, F. ARFUSO, F. TOSTO, G. PICCIONE: Promjene biokemijskih pokazatelja u preponskih konja za vrijeme simuliranih skokova. Vet. arhiv 84, 143-152, 2014.

SAŽETAK

Prosuđivane su promjene određenih biokemijskih pokazatelja i serumskih elektrolita nakon simuliranog preponskog jahanja u sedam talijanskih jahačkih konja. Izmjerena je frekvencija bila, koncentracija laktata, alkalne fosfataze, aspartat aminotransferaze, alanin aminotranferaze, kreatin kinaze, gama-glutamiltransferaze, laktat dehidrogenaze, zatim kreatinina, ureje, ukupnog bilirubina, glukoze, klorida, kalija i natrija u serumu za vrijeme odmora, nakon zagrijavanja, neposredno nakon simuliranog preponskog skakanja i 45 minuta nakon završetka vježbe. Analiza varijance ponavljanih mjerenja pokazala je znatan utjecaj vremena uzorkovanja ($P<0,05$) na frekvenciju bila, koncentraciju laktata, alkalne fosfataze, aspartat aminotransferaze, alanin aminotransferaze, kreatin kinaze, gama-glutamiltransferaze, kreatinina, glukoze i kalija. Rezultati upućuju na zaključak da poznavanje biokemijskih promjena za vrijeme simuliranog testa skakanja može pridonijeti boljoj procjeni elektrolitičkih i metaboličkih poremećaja tijekom natjecanja.

Ključne riječi: elektroliti, biokemijski pokazatelji, konj, preponsko jahanje