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Original research paper

CHANGES IN THE CLOTTING TIMES AND FIBRINOGEN CONCENTRATIONS IN HORSES DURING A SHOWJUMP

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Summary: Changes in the clotting time and fibrinolytic activity of 5 horses were assessed during a showjumping event. Venous blood samples were collected at rest prior to the trial, immediately after it and again 30 min. and 60 min. later. All the samples were immediately centrifuged at 2600 rpm for 10 min. and a Clot 2 coagulometer (SEAC, Italy) was used to assess the Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT), Thrombin Time (TT) and the fibrinogen levels of the obtained plasma. The only statistically significant differences observed during the analysis of the results were in the aPTT and fibrinogen values. Bonferroni's multiple comparison test showed that there was a statistically significant decrease ($P < 0.05$) in the aPTT immediately after the trial. It also showed that there were statistically significant decreases in the levels of fibrinogen 30 min. ($P < 0.001$) and 60 min. ($P < 0.05$) after the trial compared to those at rest, and 30 min. ($P < 0.01$) after the trial in comparison with those immediately after the trial. The results suggest an increase in blood coagulability similar to the human athlete.

Key words: coagulation; clotting time; fibrinogen; horse; physical exercise

Introduction

The effects of exercise on blood clotting and fibrinolysis have been extensively investigated. However, the extent of fibrinolysis and fibrinogenolysis after the fibrinolytic activation that occurs with exercise is still unclear.

While some investigators found increased levels of fibrin degradation products (FDPs) and evidence of fibrinogen degradation associated with exercise (1), others did not (2, 3). This could be due to differences in the duration, strenuousness and types of exercise or in the different assay methodologies.

Hunter (4) was among the first to report that the blood of animals that had been run to death was incoagulable, suggesting that exercise affects haemostasis. Fibrinolytic activity is also enhanced during exercise in healthy human beings and the magnitude of this enhancement correlates with both the intensity and duration of the exercise. Increased fibrinolytic activity appears to counterbalance exercise-induced increases in coagulability (5).

The effects of physical conditioning on blood clotting and fibrinolysis have been investigated (6, 7, 8), however, how physical conditioning affects fibrinolytic activity while at rest and exercise-induced fibrinolysis remains unclear.

The purpose of this study was to determine whether clotting times and fibrinolysis in horses are affected by maximal exercise during showjumping events.

Material and methods

Five clinically healthy and traditionally trained Sella Italiana horses, with an average age and body weight of 7 years and 450 ±35 kg, were used in our study. They were fed three times a day: at 07:00 on hay, at 13:00 on concentrates and at 19:00 on both hay and concentrates.

All the subjects underwent one month of preagonistic training, composed of five 1-hourly sessions per week. These were spent walking (15 min.), trotting (25 min.), galloping (10 min.) and jumping (2 jumps). A further two sessions per

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week were dedicated to jumping, with each horse jumping 10 obstacles between 1 and 1.10 m high.

The contest was a 350-m jump trial with 14 obstacles. All the horses were subjected to a 20-minute warm-up before the trial (5 minutes of walking, 5 minutes of galloping and 10 minutes spent jumping 6 different jumps ranging from 80 to 120 cm in height).

The blood samples, which were all collected through jugular venepunctures, were taken at rest prior to the trial, immediately after the trial and again 30 and 60 minutes after the trial. Two types of vacutainer tubes (Terumo Corporation, Japan) were used to collect the samples. Using a Hemat 8 double-capillary automatic cell counter (SEAC, Italy), the blood samples collected in EDTA vacutainer tubes were assessed in order to establish the values of the following parameters: red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT).

Blood samples that were collected in vacutainer tubes that contained 3.8 % sodium citrate were immediately centrifuged at 2600 rpm for 10 min. and the Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT), Thrombin Time (TT) and the fibrinogen levels of the resulting plasma were assessed using a Clot 2 coagulometer (SEAC, Italy).

As the intragroup variance was not significant, the statistical elaboration of the data was carried out on the mean values of the clotting and haematological parameters studied.

An analysis of variance (one-way and repeated measures ANOVA) was applied in order to evaluate the statistically significant differences between the experimental conditions (at rest *vs* immediately after the trial, 30 min. after the trial and 60 min. after the trial; immediately after the trial *vs* both 30 min. and 60 min. after the trial; and 30 min. after the trial *vs* 60 min. after the trial). If ANOVA showed an acceptable level of significance ($P < 0.05$) then Bonferroni's test was applied for a post hoc comparison.

Results

Tables 1 and 2 show the mean values of both the clotting (PT, aPTT, TT and fibrinogen) and the haematological parameters (RBC, WBC, Hb, Hct, MCV, MCH, MCHC and PLT), as well as the relative standard deviations, standard errors and statistically significant differences, of blood samples that were obtained from 5 horses that participated in an official showjumping event. The samples were taken under four different experimental conditions: at rest, immediately after the trial, 30 min. after the trial and again 60 min. after the trial.

Figure 1 shows the variations in the clotting parameters (PT, aPTT and TT) observed under those conditions.

Figure 2 is a scatter graph that shows the fibrinogen mean values obtained under the four experimental conditions.

After analysing the results, the only statistically significant differences we observed among the clotting parameters considered were those for aPTT ($F(3, 12) = 5.59$, $P < 0.01$; ANOVA for repeated measures) and fibrinogen ($F(3, 12) = 14.04$, $P < 0.0003$; ANOVA for repeated measures).

Bonferroni's multiple comparison test showed that there was a statistically significant decrease in aPTT ($P < 0.05$) immediately after the trial. It also showed that there were statistically significant decreases in fibrinogen both at 30 min. ($P < 0.001$) and 60 min. ($P < 0.05$) after the trial when compared to the at-rest values, and at 30 min. ($P < 0.01$) after the trial when compared to the values immediately after the trial. We observed statistically significant differences in the following haematological parameters: RBC ($F(2, 10) = 63.66$, $P < 0.0001$; ANOVA for repeated measures), WBC ($F(2, 10) = 13.72$, $P < 0.001$; ANOVA for repeated measures), Hb ($F(2, 10) = 62.54$, $P < 0.0001$; ANOVA for repeated measures), Hct ($F(2, 10) = 80.28$, $P < 0.0001$; ANOVA for repeated measures), MCH ($F(2, 10) = 36.07$, $P < 0.0001$; ANOVA for repeated measures), MCHC ($F(2, 10) = 127.6$, $P < 0.0001$; ANOVA for repeated measures) and PLT ($F(2, 10) = 14.02$, $P < 0.001$; ANOVA for repeated measures).

When Bonferroni's multiple comparison test was applied, RBC showed a statistical increase immediately after the trial ($P < 0.001$) as well as 30 min. after the trial ($P < 0.05$) compared to the at-rest value, and a statistical decrease 30 min. after the trial ($P < 0.001$) compared to the value immediately after the trial. WBC showed a statistically significant increase immediately after the trial ($P < 0.0l$) compared to the at-rest value. Hb

Table 1: Mean values of the clotting parameters, together with the relative standard deviations, standard errors and statistically significant differences, of blood samples that were obtained under different experimental conditions from 5 horses that participated in an official showjumping event

PARAMETER	Experimental conditions											
	Rest			Trial			After 30 min.			After 60 min.		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
PT(sec)	10.90	0.43	0.19	10.45[a]	0.15	0.07	10.66	0.09	0.04	10.50	0.16	0.08
aPTT(sec)	42.76	3.39	1.52	34.44	4.67	2.01	38.58	3.30	1.47	40.50	1.52	0.68
TT(sec)	20.34	1.98	0.88	21.03	2.51	1.12	20.26	1.27	0.57	20.28	2.09	0.94
Fibrinogen (mg/dl)	174.90	4.79	2.14	168.40	4.93	2.20	155.80[b]	4.27	1.91	162.60[a]	3.36	1.50

Explanation: a = P < 0.05 *vs* rest
b = P < 0.001 *vs* rest

Table 2: Mean values of the haematological parameters, together with the relative standard deviations, standard errors and statistically significant differences, of blood samples that were obtained under different experimental conditions from 5 horses that participated in an official showjumping event

PARAMETER	Experimental conditions											
	Rest			Trial			After 30 min.			After 60 min.		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
RBC (M/fiL)	6.93	0.40	0.17	9.69[a]	0.62	0.27	7.37[c]	0.44	0.20	7.28[d]	0.44	0.20
WBC (K/|iL)	6.70	0.76	0.34	8.21[b]	0.97	0.43	7.47	0.90	0.40	7.63	0.96	0.43
Hb fe/dL)	10.66	1.02	0.45	15.04[a]	1.11	0.50	11.42[a]	0.52	0.23	11.22	0.54	0.24
Hct (%)	33.04	3.41	1.53	47.90[a]	4.32	1.93	34.06[a]	1.61	0.72	33.82[d]	1.79	0.80
MCV(£L)	47.40	2.51	1.12	47.80	1.48	0.66	46.20	2.49	1.11	46.60	2.19	0.98
MCH(pg)	15.34	0.64	0.29	15.04[a]	0.60	0.27	15.54[a]	0.89	0.40	15.42	0.87	0.39
MCHC (g/dL)	32.28	0.29	0.13	31.46[a]	0.71	0.32	33.54[c]	0.40	0.18	33.12	0.34	0.15
PLT(K/nL)	136.40	39.78	17.79	147.80[b]	22.90	10.24	133.20[a]	12.05	5.39	138.80	18.58	8.31

Explanation: a = P < 0.001 *vs* rest b = P < 0.01 *vs* rest
c = P < 0.05 *vs* rest d = P < 0.001 *vs* trial

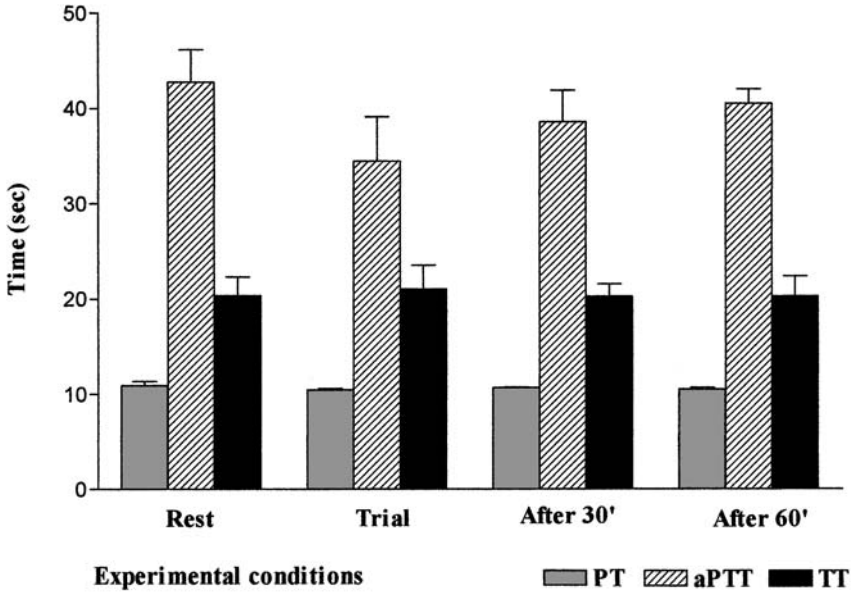

Figure 1: Variations in the clotting parameters (PT, aPTT and TT) of blood taken from 5 horses under different experimental conditions during an official showjumping event

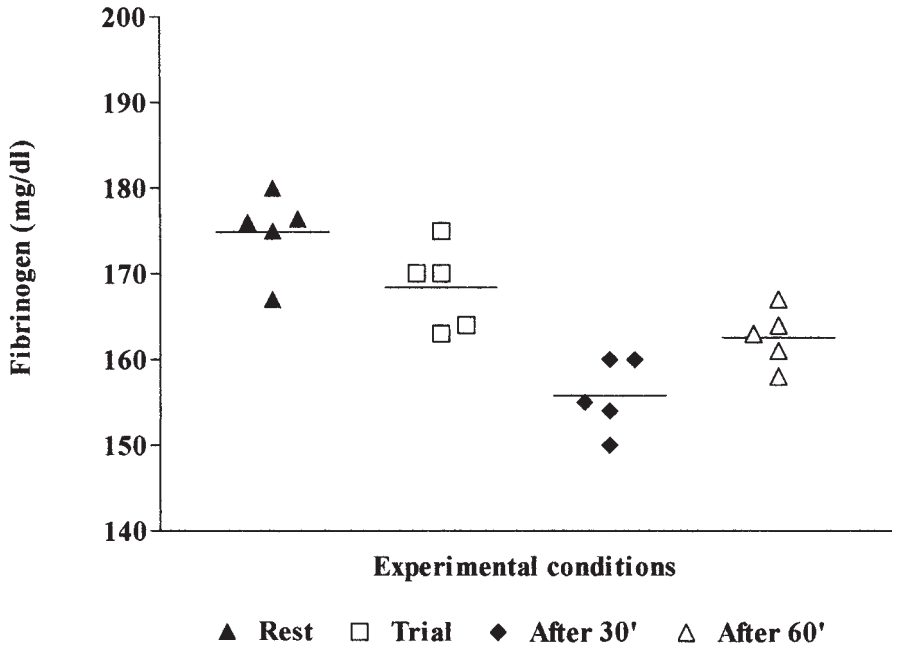

Figure 2: Scatter graph of the mean values of fibrinogen obtained from blood taken under different experimental conditions from 5 horses during an official showjumping event

showed a statistically significant increase immediately after the trial ($P < 0.001$) compared to the at-rest value and a statistical decrease 30 min. after the trial ($P < 0.001$) compared to the value immediately after the trial. Hct showed statistically significant increases immediately after the trial ($P < 0.001$) and again 30 minutes later ($P < 0.0l$) compared to the at-rest value, and a statistical decrease 30 min. after the trial ($P < 0.001$) compared to the value immediately after the trial. MCH showed statistically significant decreases immediately after the trial ($P < 0.001$) and 30 minutes later ($P < 0.001$) in comparison with the at-rest value. MCHC showed a statistically significant decrease both immediately ($P < 0.001$) and 30 min. after the trial ($P < 0.001$) compared to the at-rest value and a statistical increase 30 min. after the trial ($P < 0.01$) compared to the value immediately after the trial. PLT showed a statistical increase immediately after the trial ($P < 0.01$) and again 30 minutes later ($P < 0.05$) in comparison with the at-rest value.

Discussion

After analysing the results, the only statistically significant differences we observed among the clotting parameters considered were the aPTT and fibrinogen values obtained from the samples taken immediately after the trial; this decrease could have been caused by the type of exercise undertaken. Changes to the haemo-clotting balance have been recorded in the horse (9, 10, 11, 12), the cat (13), and in man (14).

In man it has been shown that, contrary to efforts of long duration, submaximal efforts do not involve changes in aPTT and PT (14).

Fibrinolytic activity is also enhanced during exercise in healthy human beings and the magnitude of this enhancement correlates with both the intensity and duration of the exercise (15, 16). However, the mechanism that shortens the blood clotting parameters after physical exercise remains unclear. The increased blood coagulability of horses, which is similar to that of human athletes, could represent the starting point of critical circulatory disorders such as disseminated intravascular coagulation (DIC), which is found frequently in athletic horses. Thus, the haemo-coagulative factors of athletic horses should be constantly monitored, particularly when they are subject to training and exacting competitions (9).

The haemochromatic changes were within the normal pattern for an athlete horse as has been described by several authors; these changes can be caused by exercise-induced splenic contractions (17). In fact, Persson et al. demonstrated that the spleen is the only reservoir for red blood

cells in the horse (18). Even though there were statistically significant differences between the before-and-after-trial Hct values, we did not take the influence of haemo-concentrations into account in our results as the Hct values were within the normal physiological range. The knowledge gained through this study of the clotting and haematological parameters in the athlete horse has enabled us to better understand, from a clinical point of view, the alterations in the haemostatic processes in horses during physical exercise.

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SPREMEMBE ČASA STRJEVANJA KRVI IN KONCENTRACIJE FIBRINOGENA PRI KONJIH MED PRESKAKOVANJEM OVIR

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Povzetek: Proučili smo spremembe časa strjevanja krvi in fibrinolitične dejavnosti pri petih konjih med preskakovanjem ovir. Vzorce venozne krvi smo odvzeli pred tekmovanjem, takoj po tekmovanju, 30 minut po tekmovanju in 60 minut po tekmovanju. Vse vzorce smo takoj po odvzemu centrifugirali 10 minut pri 2600 obratih na minuto. Pri dobljeni plazmi smo s koagulometrom (SEAC Clot 2) določili protrombinski čas (PT), aktivirani parcialni tromboplastinski čas (aPTT), trombinski čas (TT) in vrednost fibrinogena. Pri analizi dobljenih rezultatov smo statistično značilne razlike zaznali samo za aPTT in fibrinogen. Bonferronijev test za primerjavo več vzorcev je pokazal statistično značilno znižanje ($P < 0,05$) aPTT takoj po tekmovanju. Fibrinogen je v primerjavi s časom pred tekmovanjem izrazito padel po 30 minutah ($P < 0,001$) in po 60 minutah ($P < 0,05$), v primerjavi s časom takoj po tekmovanju pa po 30 minutah ($P < 0,01$). Rezultati kažejo na povečano sposobnost koagulacije krvi, podobno kot je znano pri ljudeh - športnikih.

Ključne besede: koagulacija; čas strjevanja krvi; fibrinogen, konj, fizična aktivnost