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Changes in blood coagulation induced by exercise training in young athletic horses

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ABSTRACT

Prothrombin Time (PT), Activated Partial Prothrombin Time (APTT), Fibrinogen concentration (Fbg) and Platelet number (Plt) were evaluated in 20 young athletic horses during a training program. A standardized exercise test (SET) was performed every month for three months. The V4 variations (the speed, in m/min, reached at the blood lactate concentration of 4 mmol/l) obtained for each test were calculated to assess the effect of training program on athletic performance. Blood samples were collected at 20-day intervals over a period of 80 days from the beginning of the training program. The V4 ($P < 0.001$), PT ($P < 0.001$), APTT ($P < 0.01$), Fbg ($P < 0.01$) and Plt ($P < 0.001$) varied throughout the training period showing that the modifications of clotting mechanism in response to training period may be considered as a normal physiological response of the hemostatic system to training exercise.

1. Introduction

Hemostasis is a complex state achieved through a delicate equilibrium between coagulation and fibrinolytic cascades (Smith 2003). The hemostatic system is very important for the maintenance of blood fluid state and vascular wall integrity, and to prevent bleeding in damaged vessels. In addition, it contributes to the hemodynamic equilibrium, regulation of the blood rheology, and vascular permeability (Tikhomirova et al., 2007). In the horse, several hemostatic changes, involving platelets, coagulation and fibrinolysis have been reported after exercise (Mckeever et al., 1990; Smith et al., 1997; Kingston et al., 2002; Piccione et al., 2004a,b, 2010; Giordano et al., 2010). In particular, blood coagulation, which occurs by means of a dynamic interaction of platelets, plasma and blood vessel endothelium, has been shown to be influenced by exercise (Prisco et al., 1994; Monreal et al., 1995; El Sayed et al., 2000). However, the data available on the effects of exercise on clotting factors differ; some researchers found that exercise increased coagulation time in healthy horses (Giordano et al., 2010), whereas others researchers found a decreased clotting time (Mckeever et al., 1990; Monreal et al., 1995; Piccione et al., 2004b), increased Von Willebrand factor (Smith et al., 1997) and decreased thrombin time (Piccione et al., 2004a) during exercise. The activation of the hemostatic process could be a protective response of the organism to exercise, and a physiological adaptation to exercise training. There are very few data on the effect of exercise training on the hemostatic system, but over the last few years a number of studies have been performed in horses subjected to acute exercise (Kingston et al., 2002; Piccione et al., 2004a,b, 2005). The present study was designed to monitor the effect of regular exercise training on Prothrombin Time (PT), Activated Partial Prothrombin Time (APTT), Fibrinogen concentration (Fbg) and Platelet number (Plt) in young athletic horses.

2. Methods

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 86/609 EEC. Twenty two-years old "rookie" Thoroughbred horses (13 stallions and 7 mares; 370 ± 20 kg body weight), were enrolled in this study. Horses were deemed healthy if they did not have a history of hemostatic abnormalities, rectal temperature, haematological parameters and some hematochemical parameters were within reference limits (Table 1). No pharmacological treatment was administered for one month prior to the onset of the study. All horses were managed similarly with individual housing, natural photoperiod, natural indoor temperature (16–18 °C) and same feeding schedule. The horses were fed with standard rations, calculated to fulfill the nutritional requirements according to INRA (Institut National de la Recherche Agronomique) specifications (Martin-Rosset, 1990). The standard ration included hay (first cut meadow hay, sun cured, late cut, 5 kg/horse day as fed), oats (4 kg/horse day as fed) and a mixed feed "oat balancer"

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Table 1
Rectal temperature and clinico pathologic parameters (mean values and standard deviations), expressed in their conventional units of measurement, of 20 Thoroughbred horses, together with reference ranges, obtained prior to beginning the study. Red Blood Cell (RBC), White Blood Cell (WBC), hemoglobin (HGB), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular hemoglobin (MCH), Mean Corpuscular hemoglobin Concentration (MCHC), Platelets (PLT), Prothrombin Time (PT), Activated partial thromboplastin time (APTT), Fibrinogen (Fbg), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Urea, Creatinine, Total Proteins (TP), Rectal Temperature (RT).

Parameters	Units of measurement	Mean values	Standard deviations	Ranges (Kaneko et al., 1997; Speirs, 1997; Thrall et al., 2004; Grondin and Dewitt, 2010)
RBC	M/μL	9.148	0.89	6.5–13
WBC	K/μL	7.82	1.14	5–12
HGB	g/dL	13.68	1.44	12–19
PCV	%	41.64	8.35	32–53
MCV	fL	45.53	3.02	34–50
MCH	pg	14.93	1.16	13–20
MCHC	g/dL	32.87	0.99	32–39
PLT	K/μL	101.10	5.33	46–194
PT	sec	12.39	1.34	10–14
APTT	sec	49.91	6.63	25–54
Fbg	g/L	1.31	0.31	1.0–4.0
AST	U/L	314.00	48.33	226–366
ALT	U/L	16.50	5.26	3–23
UREA	mmol/L	4.38	1.57	3.57–8.57
CREATININE	μmol/L	144.09	9.72	106–168
TP	g/L	74.00	7.50	58–87
RT	°C	37.69	0.20	37–39

Table 2
Standard training program for all horses.

GAIT	Duration	Speed
First and fourth day		
Walk	10 min	100 m/min
Trot	20 min	200 m/min
Canter	6 min	350 m/min
Walk	10 min	100 m/min
Second and fifth day		
Walk	15 min	100 m/min
Trot	20 min	200 m/min
Gallop	3 min	800 m/min
Walk	10 min	100 m/min
Third and sixth day		
Walk	15 min	100 m/min
Trot	8 min	300 m/min
Walk	15 min	100 m/min

(1 kg/horse day as fed). The daily ration was administered in three meals served at 8:00 AM, 12:00 PM and 5:00 PM. Diet composition was 87% dry matter and 9.1% horse digestible protein (MADC), 12.1% crude protein, 20.7% crude fiber, 3.4% ether extract and 0.80 Unité Fourragère Cheval 7 kg on dry matter basis. Water was available *ad libitum*. All horses were subjected to a standard training program that was carried out on a 2200-meter track six days a week, with one day of rest, for a 80-day period (Table 2). Training was performed at the same time for each horse every day. Each horse followed standardized exercise tests (Piccione et al., 2004a; Kedzierski et al., 2009). before the beginning of the training program (T0), and at 30 days (T30), 60 days (T60) and 90 days (T90) after T0, in order to evaluate athletic performance during the training period. The standardized test consisted of a 10-min warm up, of 10 min of slow trot (4.4 ± 0.3 m/s) followed by three stages 1200 m each, at increasing speeds (400, 500 and 600 m/min, respectively), separated by a time interval (about 1 min) necessary for blood collection. During the test, the heart rate was measured with a Polar® S610i™ sensor (Polar Electro Oy, Kempele, Finland) and the speed was measured by means of a Garmin® Forerunner™ 201 (Garmin Ltd., Romsey, U.K.) (Aerts et al., 2008). A blood sample for immediate assessment of lactate concentration was collected at rest and at the end of each stage by jugular venipuncture and it was analyzed with a portable blood lactate analyzer (Accutrend Lactate, Roche Diagnostic). For each horse, the speed at blood lactate concentration 2–4 mmol/L (V2 and V4) and HR 200 beats/min (V200), respectively, were calculated by exponential analysis (Valette et al., 1991) and linear regression (Dubreucq et al., 1995). Because our purpose was not to provide guidance on training, but to verify its effect on performance, we relied heavily on V4 as an index of the effect of the training on aerobic capacity (Couroucé et al., 1997). Blood samples were drawn at 6:00 AM, every 20 days for 80 days (T0-T80) to evaluate the PT, APTT, fibrinogen concentration and platelet count. Blood samples were collected from the jugular vein, paying attention to avoid unnecessary manipulation of the sampling site, which could result in activation of coagulation. Blood samples were collected into 2 ml vacutainer tubes with ethylenediamine tetraacetic acid (EDTA) for measurement of the platelet count with an automated hematology analyzer (HecoVet, SEAC, Florence, Italy), and into 3.6 ml vacutainer tubes containing 3.8% sodium citrate (1 part citrate and 9 parts blood) to evaluate the PT, APTT, and fibrinogen concentration by means of a coagulation analyzer (Clot 2, SEAC, Florence, Italy). Blood samples anticoagulated with citrated sodium were centrifuged at 1500*g* for 15 min within 15 min following collection (Casella et al., 2009b). All samples were assayed in duplicate by the same person each time. Samples exhibited parallel displacement to the standard curve; the intra-assay coefficient of variation was <7% for all parameters measured and the inter-assay coefficient of variation was <9% for all parameters measured.

2.1. The PT test

Prothrombin time was automatically assessed on citrated plasma by means of a standard kit (Seac-Radim Company, Germany) made especially for the SEAC Clot 2 coagulometer. The PT kit was based on the assay principle that the addition of an adequately calcified amount of tissue factor (factor III) to citrated plasma activates factor VII, which induces formation of a stable plug. The assay procedure was performed by placing 200 μl of tissue factor (PT reagent) in a test tube preheated to 37 °C and subsequently adding 100 μl of citrated plasma. Upon the addition of test plasma, a stopwatch was started automatically and the clotting time was measured. The time in seconds from plasma-reagent mixing to visual clot formation was defined as the PT.

2.2. The APTT test

Activated partial thromboplastin time was automatically assessed on citrated plasma by means of a standard kit (Seac-Radim Company, Germany) made especially for the SEAC Clot 2 coagulometer. The APTT kit was based on the addition of a platelet substitute (phospholipids and ellagic acid as a soluble activator) and calcium chloride, which induced the formation of a stable plug. The assay procedure was performed by placing 100 μl of citrated plasma and 100 μl of APTT reagent (preheated to 37 °C) in a test tube preheated to 37 °C, followed by an additional incubation for 3 min at 37 °C, and then adding 100 μl of calcium chloride. Upon the addition of calcium chloride, a stopwatch was started automatically and the clotting time was measured. The time in seconds from calcium chloride addition to visual clot formation was defined as the APTT.