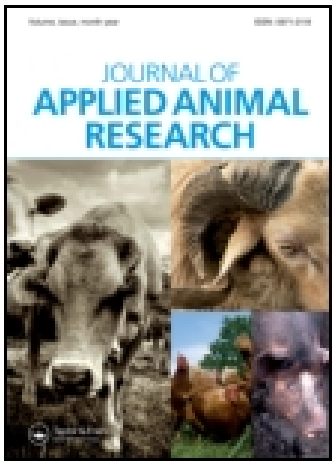

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Exercise-induced Modifications on Haematochemical and Electrophoretic Parameters During 1600 and 2000 Meters Trot Races in Standardbred Horses

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Abstract

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The aim of the present study was to evaluate the modifications of some haematochemical and electrophoretic parameters during two different workloads in Standardbred horses. Ten clinically healthy horses were divided into two equal groups, taking part in an official trot race at 1600 or 2000 meters course, respectively. Blood samples were collected from each horse at rest, after warm up, 0, 30 and 60 min after the end of the race. Serum concentrations of albumin, globulins, total proteins, total cholesterol, triglycerides, glucose, hydroxybutyrate, urea, creatinine, creatine kinase (CK), glutamate pyruvate transaminases (GPT) and glutamate oxalacetate transaminases (GOT) were assessed. Exercise increased triglyceride, glucose, creatinine and CK and decreased GOT which tended to reach rest level 60 min after exercise. Globulins were affected by exercise but there was no set pattern.

Key words: Athletic horse, haematochemical parameters, electrophoretic parameters, trot races, workload.

Introduction

Horses have a great capacity for physical work. They are exposed to chronic prolonged stress, such as daily training and frequent races during their active lifespans (Passantino *et al.*, 2005).

Many studies have been performed to evaluate changes in physiological and biochemical parameters in Thoroughbred (Piccione *et al.*, 2007b; c) and Standardbred racehorses (Piccione *et al.*, 2007d). Many authors studied the adaptive processes to exercise in horse and they noted that the horse have a regulatory system which responds in a complex manner to stress as exercise (Coenen *et al.*, 2005). Other studies have been carried out with the purpose of underlining the pattern of some haematochemical and haematological parameters during training and physical exercise (Piccione *et al.*, 2003).

Considering that, from a physiology effort point of view, correct management is based on knowledge of the metabolic and functional

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processes involved in the particular athletic discipline. Haematochemical and electrophoretic parameters modifications due to two different workloads, 1600 meters and 2000 meters official races, were studied in Standardbred horses.

Materials and Methods

Ten clinically healthy gelding Standardbred horses from the same horse training centre, (5±3 years, 430±30 kg) were traditionally fed with hay and a mix of cereals (oats and barley) and water was available *ad libitum*.

All animals were trained appropriately and competed regularly in "La Favorita" racetrack (Palermo - Sicily - Italy) where they were subjected to warm up and, subsequently, to two different workloads. Horses were divided into two equal groups. The Group A took part in 1600 meters trot official race (average speed 831.4 m/min in 1'19"3 time/km) while the Group B took part in 2000 meters trot official race (average speed 831.6 m/min in 1'20"2 time/km).

Blood samples were collected through jugular venipuncture, using vacutainer tubes (Terumo Corporation, Japan) with no additive, at rest, after warm up, immediately after the race, 30 and 60 min after the end of the race, serum was separated and stored at -20C until analyses. Serum concentrations of albumin, globulins (α1-globulins, α2-globulins, β-globulins, γ-globulins), total proteins and the albumin/globulins ratio were assessed by means of automated multiparametric agarose gel electrophoresis system (Hydrasys, Sebia, France); while serum concentrations of total cholesterol, triglycerides, glucose, hydroxybutyrate, urea, creatinine, creatine kinase (CK), glutamate pyruvate transaminases (GPT) and glutamate oxalacetate transaminases (GOT) were analysed with commercially available kits by means of a UV spectrophotometer (model Slim SEAC, Firenze, Italy).

Two-way repeated measures analysis of variance (ANOVA) was applied to values. Bonferroni's test was applied for post hoc comparison and $P \leq 0.05$ was considered statistically significant. Data were analyzed using STATISTICA 5.5 (Stat Soft Inc.) software package.

Results and Discussion

The relative contribution of the protein fractions changed during the trial. At rest α1-globulins values in the two groups were quite different and this could be due to different type of training as demonstrated in horses performing submaximal exercise and horses performing maximal exercise (Hinchcliff *et al.*, 2004).

We have found a decrease in relative amount of α1-globulins after the race associated to a significant variation in the relative amount of α2-globulins, β-globulins and γ-globulins that change in a heterogenous manner (Hinchcliff *et al.*, 2004) (Table 1). Alternative mechanism by which heterogenous alterations in plasma protein fraction concentrations may occur in horse during running exercise include preferential compartmental redistribution, accelerated biosynthesis, increased degradation and bolus release. In part, the relative small molecular weight of globulins greatly facilitates transfer of this fraction into extravascular space (Coyne *et al.*, 1990). As previously observed in show jumping (Piccione *et al.*, 2007a) also 1600 and 2000 meters trot races had a significant effect on globulins probably due to enzymatic degradation of protein fractions.

Tryglicerides showed a significant increase after the race and after 30 min, which decreased after 60 min in both groups. However, although triglycerides showed the same trend during experimental period, the different workload determined significant differences of this parameter comparing the different experimental conditions. It may be due to nonesterified fatty acids (NEFAs) that, at the conclusion of a race, may provide as much as 90% of the total energy requirements; so the triglycerides increase later in the

Table 1
Average values (±SD) of electrophoretic parameters studied in Standardbred horses of Groups A and B during experimental conditions

Parameters	Groups	Experimental condition				
		At rest	After warm up	After the race	After 30 min	After 60 min
Albumin	A_B	28.0±3.30	30.20±3.90	24.10±2.90	22.00±3.80	27.80±2.80
		28.30±1.40	24.70±2.70	32.50±2.70	28.80±2.60	25.40±2.70
α 1-globulin	$A_{B\#}$	6.10±1.10	6.60±0.80	4.70±0.40*^{x}	10.40±0.50*^{xa}	8.10±0.70*^{xab}
		3.70±0.90	5.20±0.90*	4.50±0.60	4.60±0.90	3.40±0.80^{xab}
α 2-globulin	$A_{B\#}$	6.20±0.90	6.80±1.10	5.50±0.90^{x}	6.90±0.60^{a}	5.20±0.10^{xb}
		7.50±0.90	5.70±0.50*	8.40±1.20 ^{x}	6.20±1.10^{a}	7.40±1.00^{x}
β - globulin	$A_{B\#}$	7.60±0.60	8.60±0.70*	5.70±0.70*^{x}	7.10±0.70^{xa}	8.00±0.90 ^{a}
		8.00±0.70	9.70±0.70*	9.90±0.60*	8.30±1.00 ^{xa}	7.60±0.50 ^{xa}
γ - globulin	A_B	11.6±1.60	12.2±0.90	10.90±0.70	13.90±1.10*^{xa}	8.80±0.80*^{xab}
		12.20±0.90	8.60±0.70*	14.50±1.00*^{x}	11.60±1.00 ^{xa}	11.80±1.10^{xa}
Albumin/globulins	A_B	9.00±1.40	8.90±1.60	8.90±0.70	6.80±1.70	9.20±1.20
		9.30±2.00	8.90±2.20	8.70±2.20	9.7 0±2.50	8.60±1.50
Total Protein	A_B	60.40±7.60	64.60±9.30	51.00±4.00	60.60±4.40	58.40±2.90
		60.00±6.10	53.20±5.70	71.20±8.10	59.60±4.90	55.40±8.00

Significances of exercise: * *vs* at rest; ^{x} *vs* after warm up; ^{a} *vs* after the race; ^{b} *vs* after 30 min;
Significances of different workload: $^{\#}$ *vs* Group A.

exercise period than the NEFAs, probably because beside being oxidized in skeletal muscle the NEFAs act as precursors for triglycerides (Hodgson and Rose, 1994). Therefore, exercise intensity influences their utilization that affects triglycerides concentrations.

Glucose concentration significantly increased after the race (Table 2) and significantly decreased after 30 min with the same trend comparing the two groups probably because of stimulation of hepatic glycogenolysis.

Few studies have been conducted on hydroxybutyrate. It showed a significant decrease after 30 min in Group B. This modification is probably due to exercise that induces stress (Passantino *et al.*, 2005).

As previously observed, increases in both urea and creatinine are found in response to exercise (Hodgson and Rose, 1994). These traditional indices of renal function are also affected by prerenal factors, such as hemoconcentration. Additionally, creatinine increases during exercise as a result of increased phosphocreatine turnover and therefore, increases in plasma or serum creatinine cannot be used as an indication of reduced glomerular filtration rate. After exercise where there has been extensive fluid loss in the sweat, the reduction in renal blood flow and glomerular filtration rate may continue because acute renal failure is a common complication of exhaustion in athletic horse. In both groups this will be reflected by persistent elevations in urea concentrations (at rest in group A: 4.38±1.57; B: 5.31±0.38) and in creatinine concentrations (at rest in group A: 144.09±9.72; B: 137.02±12.37) for 30 min after the race (urea in group A: 4.71±1.93; B: 5.71±0.36; creatinine in group A: 157.35±6.18; B: 160.00±22.98).

Table 2
Average values (±SD) of haematochemical parameters studied in Standardbred horses of Groups A and B during experimental conditions.

Parameters	Groups	Experimental condition				
		At rest	After warm up	After the race	After 30 min	After 60 min
Total cholesterol	A_B	1.79±0.23	1.92±0.30	1.92±0.33	1.92±0.34	1.83±0.37
(mmol/l)		1.89±0.24	2.05±0.20	2.03±0.18	1.82±0.24	1.79±0.30
Triglycerides	$A_{B\#}$	0.25±0.04	0.35±0.08	0.67±0.16*^{x}	1.19±0.20*^{xa}	0.71±0.13*^{xb}
(mmol/l)		0.19±0.06	0.31±0.07	1.12±0.33*^{x}	1.78±0.30*^{xa}	1.07±0.19*^{xb}
Glucose (mmol/l)	A_B	4.49±0.47	5.58±0.59	8.94±0.58*^{x}	7.68±0.41*^{xa}	6.46±0.53*^{ab}
		4.39±0.66	5.19±0.70	9.96±0.87*^{x}	6.80±0.78*^{xa}	5.53±0.63*^{ab}
Hydroxybutyrate	A_B	0.10±0.02	0.10±0.01	0.10±0.01	0.09±0.01	0.08±0.01
(mmol/l)		0.11±0.02	0.10±0.01	0.09±0.01	0.08±0.02*^{x}	0.09±0.02
Urea (mmol/l)	A_B	4.38±1.57	4.44±1.58	4.18±1.28	4.74±1.82^{x}	4.71±1.93^{x}
		5.31±0.38	5.41±0.25	5.81±0.40*	5.74±0.34*	5.71±0.36
Creatinine (μmol/l)	A_B	144.09±9.72	151.16±7.07	144.97±4.42	169.72±5.30*^{xa}	157.35±6.18*^{a}
		137.02±12.37	151.16±12.37*	159.12±22.98*	166.19±19.44*^{x}	160.00±22.98*
CK (U/l)	$A_{B\#}$	181.00±59.76	230.20±38.23	379.60±57.38*^{x}	400.60±60.59*^{x}	378.80±58.06*^{x}
		151.40±44.04	177.60±47.13	267.60±52.49*^{x}	288.20±43.21*^{x}	320.00±59.16*^{x}
GPT (U/l)	A_B	23.20±6.26	25.40±7.26	29.80±5.54*	21.20±6.37^{a}	20.80±6.41^{a}
		17.00±5.19	22.80±6.87*	27.00±7.76*	27.60±5.12*^{x}	18.60±5.89^{ab}
GOT (U/l)	A_B	314.00±48.33	303.20±41.08	196.40±30.47*^{x}	196.00±40.07*^{x}	234.60±46.16*
		291.20±69.40	314.20±72.83	158.20±58.51*^{x}	184.20±59.25*^{x}	225.80±42.60^{x}

Significances of exercise: * *vs* at rest; ^{x} *vs* after warm up; ^{a} *vs* after the race; ^{b} *vs* after 30 min
Significances of different workload: $^{\#}$ *vs* Group A.

It is well known that activities of various enzymes, particularly of creatine kinase and glutamate pyruvate transaminases, increase following muscle damage or strenuous muscular exercise (Balogh *et al.*, 2001).

Generally speaking modest increases in muscle-derived enzymes are found in the plasma or serum in response to both low- and high- intensity exercise. These increase have been suggested to reflect increase in mitochondrial membrane permeability rather than muscle damage (Hodgson and Rose, 1994). Thus both exercise duration and intensity are important in the increase in the muscle-derived enzymes that occur during exercise. In trotter horse, subjected to official trot races of 1600 and 2000 meters, the different exercise intensity did not determine a significant effect of different workload.

Only limited studies have been carried out investigating alterations in other enzymes with exercise. Our results showed that GOT decreased after the race and after 30 min while it increased after 60 min as previously observed in jumping horses (Assenza *et al.*, 1996). These modifications probably are due to stress induced from the exercise, in fact the GOT concentration began to increase again later.

In conclusion we can affirm that the modifications in the concentration of parameters studied suggest that short-term exercise represents a very important moment in the life of the athletic horse; in fact in trotter's races as in other discipline, variations observed on some haematochemical and electrophoretic parameters may be due to both the exercise and different workload in trot races.

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जी.पीकिओन, एस. कैसेल्ला, सी. जीयानेट्टो, वी. मोन्टेबर्डे, वी. फिर्रेन्टेल्ली। मानक संवर्धित अश्वों में 1600 और 2000 मीटर दुलकी दौड़ के समय रुधिर-रसायनी और इलेक्ट्रोफोरेटी प्रामकों में श्रम प्रेरित परिवर्तन।

इस अध्ययन का उद्देश्य मानक संवर्धित अश्वों में दो भिन्न गति के कार्य भार का कुछ रुधिर-रसायनी और इलेक्ट्रोफोरेटी प्रामकों में श्रम प्रेरित परिवर्तन का मूल्यांकन करना था। दस स्वस्थ अश्वों को 2 समान वर्गों में बांट कर 1600 और 2000 मीटर की राजकीय दुलकी दौड़ में भाग लेने के लिए चयनित किया गया। सभी अश्वों से विश्राम के समय, सक्रिय करने के बाद तथा दौड़ समाप्ति के 30 और 60 मिनट बाद रुधिर के नमूने लिए गये। सीरम में एल्ब्युमिन, ग्लोबुलिन, प्रोटीन, सकल कोलेस्ट्राल, ट्राइग्लीसराइड, ग्लूकोज, हाइड्राक्सीब्युटारेट, यूरिया, क्रीएटिन, क्रीएटिन काइनेज (सीके), ग्लुटामेट पाइरुवेट ट्रान्सएमिनेज (जीपीटी) और ग्लुटामेट अक्जाल एसिटेट ट्रान्सएमिनेज (जीओटी) का विश्लेषण किया गया। श्रम से ट्राइग्लीसराइड, ग्लूकोज, क्रीएटिन और सी के में वृद्धि तथा जीओटी में कमी हुई जो कि 60 मिनट विश्राम के बाद सामान्य हो गये। श्रम से ग्लोबुलिन प्रभावित हुए परन्तु उनकी स्थिर रूपरेखा नहीं थी।