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Modification of Some Haematological and Haematochemical Parameters in Horse During Long Distance Rides

[1]G. Piccione, [2]I. Vazzana, [1]C. Giannetto, [3]M. Gianesella and [2]V. Ferrantelli
[1]Dipartimento di Morfologia, Biochimica, Fisiologia e Produzione Animale,
Sezione di Fisiologia Veterinaria, Facoltà di Medicina Veterinaria,
Università Degli Studi di Messina, Italy
[2]Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy
[3]Dipartimento di Scienze Cliniche Veterinarie, Facoltà di Medicina Veterinaria,
Università Degli Studi di Padova, Italy

Abstract: The aim of this study was to evaluated the effect of the low-intensity long-lasting trial on some haematological and haematochemical parameters during an international endurance race. Six clinically healthy and regularly trained Sella Italiana mares were used. On blood sample collected at rest, at 30 km and 30 after the trial, haematological (Red Blood Cell, Haemoglobin, Haematocrit; Mean Corpuscolar Volume, Mean Corpuscolar Hemoglobin, Mean Corpuscolar Hemoglobin Concentration, White Blood Cell and Platelets) and haematochemical parameters (Glucose, Aspartate aminotransferase, Total protein and protein fraction (albumin, α_1-globulins, α_2-globulins, β-globulins and γ-globulins), Triglycerydes, Cholesterol, Creatinine, Urea, Potassium, Sodium and Chloride) were assessed. One way repeated ANOVA showed a statistical significant effect of low-intensity long-lasting trials on the following parameters: Red Blood Cell ($p \leq 0.009$), Haematocrit ($p \leq 0.031$), Mean Corpuscolar Hemoglobin Concentration ($p \leq 0.013$), White Blood Cell ($p < 0.0001$), Platelets ($p < 0.0001$), α_1-globulins ($p \leq 0.038$) and γ-globulins ($p \leq 0.009$), Creatinine ($p \leq 0.023$) and Potassium ($p \leq 0.012$). Our results confirm the effects of endurance trial on horse metabolism, underlining that haematological and haematochemical profiles could be an useful index for the prevention of many typical diseases of the athlete horse other than in the assessment of the fitness of the endurance horse.

Key words: Haematological parameters, haematochemical parameters, low-intensity long-lasting trials, horse

INTRODUCTION

The assessment of the state of fitness of horses in training and the state of fatigue during and after competition is a very subjective matter, particularly where endurance horses are concerned (Kerr and Snow, 1983). In sport exercise physiology, the word endurance defines the physical and mental capacity to withstand fatigue (Weineck, 1990). Endurance competitions can be classified as low-intensity long-lasting trials; they are extremely difficult from a metabolic point of view, causing the elimination of 7.2% of horses starting in international races, but some are retired for the same reasons between two veterinary gates during the race, while others have problems after the final veterinary examination (Bergero *et al*., 2005a). Physical work capacity of the horse is a function of both maximal aerobic power and anaerobic capacity (McMiken, 1983). Both aerobic and anaerobic a shortage of fuel, especially depletion of glycogen stores (Essén-Gustavsson *et al*., 1984, 1991).

Corresponding Author: Giuseppe Piccione, Dipartimento di Morfologia, Biochimica, Fisiologia e Produzioni Animali, Facoltà di Medicina Veterinaria, Sezione di Fisiologia Veterinaria, Università degli Studi di Messina, Polo Universitario dell Annnunziata, Postal Code 98168, Messina, Italy Tel: +39903503584 Fax: +39903503975

Low glucose concentration limits the glucose availability to the central nervous system. During endurance exercise, factors not related to substrate supply may be more important in the development of fatigue. These include the loss of electrolyte in sweat, which may disturb the neuronal control of muscle concentrations, loss of water in sweat that may hamper oxygen and substrate supply via the circulation (Pösö *et al*., 2004). With ensuing sweat losses of water and electrolytes occurring at elevated rates, then a slowly progressing metabolic alkalosis develops (Rose *et al*., 1979). The factors determining capability of a horse for an effort are efficiency of oxygen uptake and its distribution speed to working muscle. Blood lactate represents the index of performance capacity in endurance horses (Hodgson and Rose, 1994), while total protein level is the helpful index in the assessment of the physiological state of an animal (Szarska and Cuber, 1994). Measurements of total protein, albumin, globulins can provide an index of hydration status, as well as indices of infection, inflammation, increase protein loss, or decreased protein production (Rose and Hodgson, 1994). It is generally accepted that during prolonged exercise there is a net break down of whole-body protein accomplished by a decrease in the rate of protein synthesis and an increase in the rate of protein degradation in the liver (Dohm, 1986)

Studies in humans and in dogs have indicated that protein is an unimportant substrate during exercise the contribution of protein energy expenditure in horses during exercise is unknown, but it has generally been assumed that carbohydrates and fat oxidation predominate (Pösö *et al*., 2004); although under some circumstances amino acid oxidation may account for up to 5-10% of energy expenditure (Spriet and Peters, 1998). For the sport horse, the engagement of protein metabolism during exercise and possible role of some amino acids in the onset of fatigue is suggestive, but mostly unexplored (Assenza *et al*., 2004). Some studies were carried out about amino acid modification during different kinds of exercise (Bergero *et al*., 2003; Assenza *et al*., 2004; Bergero *et al*., 2005a, b) and about total protein modification (Deldar *et al*., 1982; Ahkenazi and Epshtein, 1998), but little is known about protein fractions.

Considering that, from a physiology effort point of view, correct management is based on knowledge of the metabolic and functional processes involved in the particular athletic discipline, haematological and haematochemical modifications during an international endurance race were studied.

MATERIALS AND METHODS

Six Sella Italiana mares, clinically healthy and regularly trained, with a body weight of 400±20 kg, were used. Horses were fed hay and a mixture of cereals (oats and barley), three times a day (07.00, 12.00 and 17.00) and water *ad libitum*. Before the start of the study, all the subjects underwent a heart exam, respiratory auscultations and routine haematology and plasma biochemistry at rest. After evaluation of these parameters only clinically healthy animals were chosen as study materials. All six mares took part in an International Endurance Competition- CEI (distance of 30 km) in Sicily (Italy) in June 2006. Blood samples were collected at rest, at the check point (30 km after the start) and 30 min after the trial, through an external jugular venipuncture using two vacutainer tubes (Terumo Corporation, Japan) for each subject, one with K_3-EDTA and one with no additive.

On blood samples, collected using vacutainer tubes (Terumo Corporation, Japan) with K_3-EDTA, glucose was assessed by means of Blood Glucose Meter (Glucotrend 2, Roche), immediately after the collection while an automated haematology analyzer (Celly-Dyn 3700, Abbott) was used to assess the following parameters: Red Blood Cell (RBC), Haemoglobin (Hb), Haematocrit (Hct); Mean Corpuscolar Volume (MCV), Mean Corpuscolar Hemoglobin (MCH), Mean Corpuscolar Hemoglobin Concentration (MCHC), White Blood Cell (WBC) and Platelets (PLT).

Blood samples, collected using vacutainer tubes (Terumo Corporation, Japan) with no additive, were centrifuged at 3000 x g for 10 min. On the obtained sera, stored at −20°C pending analysis, Aspartate aminotransferase (AST), Total Protein, Triglycerides, Cholesterol, Creatinine, Urea, Potassium, Sodium and Chloride were assessed by means of automatic analyzer (Konelab 20, Dasit).

Total protein separate fractions (albumin, α_1-globulins, α_2-globulins, β-globulins and γ-globulins) were assessed by means of an electrophoresis system (Helena Biosciences Europe-UK).

Since the intragroup variance was not significant, the statistical elaboration of data was carried out on mean values of the studied parameters. The analysis of variance (one-way and repeated measures ANOVA) was applied to evaluate the statistical significance of differences between the different experimental conditions (at rest vs immediately after the trial, at rest vs 30 min after the trial and immediately after the trial vs 30 after the trial). Where ANOVA showed an acceptable level of significance ($p<0.05$), Bonferroni's test was applied for post hoc comparison. All results were expressed as means±standard errors of the means (SEM).

RESULTS

Present results were within the physiological range for the horses (Kaneco, 1989).

ANOVA showed a statistical significant effect of low-intensity long-lasting trials on the parameters studied as follow: RBC, $F_{(2,17)} = 7.77$, $p \leq 0.009$; Hct, $F_{(2,17)} = 4.99$, $p \leq 0.031$; MCHC, $F_{(2,17)} = 6.91$, $p \leq 0.013$; WBC, $F_{(2,17)} = 32.31$, $p<0.0001$; PLT, $F_{(2,17)} = 24.39$, $p<0.0001$; α_1-globulins, $F_{(2,17)} = 4.58$, $p \leq 0.038$; γ-globulins, $F_{(2,17)} = 7.61$, $p \leq 0.009$; Creatinine, $F_{(2,17)} = 5.61$, $p \leq 0.023$; Potassium, $F_{(2,17)} = 6.93$, $p \leq 0.012$.

RBC and Hct showed a statistical significant increase after trial vs rest ($p<0.01$ and $p<0.05$, respectively, Bonferroni's test) and did not return to the basal level 30 min after trial. MCHC showed a statistical significant decrease after trial and 30 min after trial vs rest ($p<0.05$, Bonferroni's test). WBC showed a statistical significant increase after trial and 30 min after trial vs rest ($p<0.001$, Bonferroni's test). PLT showed a statistical significant increase after trial vs rest ($p<0.001$, Bonferroni's test) and did not return to the basal level 30 min after trial ($p<0.01$, Bonferroni's test). α_1-globulins showed a statistical significant decrease after race vs rest ($p<0.05$, Bonferroni's test) and almost went back to the rest level within 30 after race. While γ-globulins showed a statistical significant increase 30 after race vs rest ($p<0.01$, Bonferroni's test). Creatinine showed a statistical significant increase after 30 vs rest ($p<0.05$, Bonferroni's test). On the contrary, Potassium showed a statistical significant decrease after 30 vs rest ($p<0.05$, Bonferroni's test) (Table 1 and 2).

Table 1: Haematological and haematochemical parameters mean values (±SEM) expressed in their conventional units, together with their statistical differences, obtained on the different experimental conditions in 6 Sella Italiana mares during an International Endurance Competition of 30 km

	Experimental conditions		
Parameters	Rest	After trial	After 30'
Haematological			
RBC (10^6 mm^{-3})	7.60±0.160	8.90±0.37♦	8.35±0.38
Hb (g dL^{-1})	12.36±0.170	14.08±0.78	13.38±0.66
Hct (%)	28.75±0.510	33.51±1.90*	31.72±1.63
MCV (fl)	38.32±.0680	38.45±0.50	38.40±0.50
MCH (pg)	16.48±0.330	16.20±0.28	16.23±0.28
MCHC (g L^{-1})	43.03±0.330	42.17±0.25*	42.28±0.22*
WBC (10^3 mm^{-3})	8.23±0.500	11.72±0.65°	13.18±0.84°
PLT (10^3 mm^{-3})	140.00±10.07	195.70±10.77°	181.70±11.39
Haematochemical			
Glucose (mg dL^{-1})	51.33±3.93	39.83±8.41	35.17±4.48
AST (UI L^{-1})	339.70±38.72	385.60±9.12	397.60±10.28
Tryglycerides (mg dL^{-1})	19.50±2.070	29.00±4.88	26.00±7.65
Cholesterol (mg dL^{-1})	77.17±2.380	79.50±2.65	81.33±2.75
Creatinine (mg dL^{-1})	1.17±0.080	1.46±0.03	1.50±0.09*
Urea (mg dL^{-1})	32.33±1.220	34.50±2.01	38.67±3.68
Potassium (mmol L^{-1})	3.73±0.300	3.46±0.19	2.71±0.06*
Sodium (mmol L^{-1})	133.80±07000	132.50±0.67	132.5±1.110
Chloride (mmol L^{-1})	92.00±0.570	89.33±0.61	88.50±1.78

Significance: *: vs rest $p<0.05$; ♦: vs rest $p<0.01$; °: vs rest $p<0.001$

Table 2: Total protein and protein fractions mean values (±SEM) expressed in their conventional units, together with their statistical differences, obtained on the different experimental conditions in 6 Sella Italiana mares during an International Endurance Competition of 30 km

	Experimental conditions		
Parameters	Rest	After trial	After 30'
Total protein (g L^{-1})	6.58±0.17	6.96±0.21	7.05±0.24
Albumin (g L^{-1})	42.55±1.02	41.09±1.22	39.56±1.64
α_1-globulins (%)	5.19±0.40	3.72±0.24*	4.46±0.30
α_2-globulins (%)	12.11±0.27	12.69±0.54	12.20±0.55
β-globulins (%)	16.07±1.92	16.49±1.64	16.20±1.63
γ-globulins (%)	23.74±1.56	25.16±2.01	26.58±1.88♦

Significance: *: vs rest $p<0.05$; ♦: vs rest $p<0.01$

DISCUSSION

Modification in haematological and biochemical values associated with low-intensity long-lasting trials reflected aerobic capacity, dehydratation, muscle damage and reduction in renal function.

RBC count and Hct showed a statistical significant increase after trial vs rest reflecting the mobilization of erythrocytes from the splenic reservoir, resulting in a greater demand for oxygen carriage (McKeever, 2004). Up to 12 L blood can be delivered allowing the equine athlete to reach a maximal aerobic capacity (Rose and Evans, 1987). While most of the Hct increase is relate to splenic erythrocytes release, there are also substantial fluid shifts out of the plasma during exercise and therefore, some of increase in Hct is due to fluid movement (Rose and Hodgson, 1994). This is also the cause of fluid losses through the sweat that, in endurance races may lead to dehydration values of 6.2% with body weight losses of 4.6% in horses (Assenza *et al*., 1999; Bergero *et al*., 2001). Change also occurred in erythrocyte, with statistical significant decrease in mean corpuscular haemoglobin concentration. WBC showed a statistical significant increase after trial and after 30 vs rest; the proportions of leukocytes change was based on the intensity and duration of exercise, as well as the degree of stress to which the horses were subjected. Long-distance, low- to moderate-intensity exercise produces a leucocytosis that results in neutrophilia and lymphopenia (Rose and Hodgson, 1994).

As previously observed in men (Kestin *et al*., 1993) platelets showed a statistical significant increase after trial vs rest and did not return to the rest level within 30 min after the trial. However, the effects of strenuous exercise on platelet function are controversial. There is evidence that equine platelet reactivity is altered by strenuous exercise. Change in platelet reactivity could impact haemostasis following exercise-induced injury and may play a role in the pathophysiology of exercise-induced pulmonary haemorrhage, that has been reported in almost all types of equine athletes (Johnstone *et al*., 1991). The significance of increase number of platelet-neutrophil aggregates in association with exercise is currently unknown and further investigation are needed (Kingston *et al*., 2002).

The relative contribution of the protein fractions separated by paper electrophoresis was changed during the trial. A statistical significant decrease in relative amount of α_1-globulins was accompanied by a statistical significant increase in the relative amount of γ-globulins. Alternative mechanism by which heterogeneous alterations in plasma protein fraction concentrations may occur in horse during acute 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 jumper horses (Piccione *et al*., 2007) also low-intensity long-lasting trials had a significant effect on α_1-globulins probably due to enzymatic degradation of protein fractions, that has been recognized during running exercise in horses and human beings (Ferguson *et al*., 1981; Bartsch *et al*., 1982). Because RBC increase greatly during exercise in the horse and several plasma protein fraction

display a rather marked affinity for the exterior RBC surface membrane, massive increases in the absolute mass of intravascular RBC may serve to sequester concentrations of certain plasma protein fractions (Coyne *et al*., 1990).

Serum Creatinine concentration increased after the trial and remained significantly high 30 min after trial. Creatinine is a metabolite of creatine, it is found in high concentrations in excitable tissues in which there is a high and fluctuating energy demand. Highest concentrations are found in skeletal muscle where it accounts for 98% of the body's total creatine pool (Sewell and Harris, 1995), so the increase in creatinine concentration is in relation to the muscular activity. Its increases during exercise is the result of increased phosphocreatinine turnover (Rose and Hodgson, 1994). Plasma creatinine concentration and production may suggest muscle damage (Irving *et al*., 1990). Strenuous exercise causes a sequential increase in free radical levels (Snaders, 1995). Oxygen free radicals have a vasoconstrictive function, leading to a reduced glomerular filtration rate by directly inactivating cyclooxygenase in the epithelial cells (Ohta *et al*., 2004). In addition to free radicals, exercise causes increased levels of endothelin, cathecolamines, angiotensin II, endotoxin, cytokines and leucotrienes (Snaders, 1995). In particular arginine and vasopressine increase in correlation with exercise duration and intensity both in horse and in man (Wade and Freund, 1990; McKeever *et al*., 1992). Their sustained elevations following exercise stimulate thirst and drinking, cause decreases in free water clearance by the kidneys and may influence the uptake of sodium and water from the colon (McKeever *et al*., 1991). The alteration in renal function is short-lived, with the return to normal range shortly after cessation of exercise (Rose and Hodgson, 1994) even if these exercise-induced mediators also may facilitate renal ischemia (Ohta *et al*., 2004) and acute renal failure (Hisanaga *et al*., 1999).

Potassium occurs mainly in the ICF and rapid release and uptake by the exercising muscle and decreases in serum potassium concentration occur quickly upon cessation of exercise (Harris and Snow, 1992). Therefore, change in serum potassium concentrations in endurance races may reflect the time of sampling relative to exercise lasting and intensity. Serum potassium concentration had the highest coefficient of variation, compared to other parameters, reflecting the potassium flow between body fluid compartments (Ecker and Lindinger, 1995).

CONCLUSIONS

The assessment of metabolism of the endurance horse is important in the prevention and in the treatment of diseases such as pulmonary hemorrhage, rhabdomyolysis and kidney failure due to lasting and intense exercise other than the evaluation of the performance of the horse.

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