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**PLASMA MALONDIALDEHYDE, BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS IN STANDARDBRED HORSES DURING A SELECTED FIELD EXERCISE TEST**

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*The aim of the present study was to determine changes in plasma malondialdehyde (MDA) concentration and haematological and biochemical profiles in 10 clinically healthy standardbred horses subjected to a selected field exercise test. Correlations between plasma MDA, the main lipid peroxidation end-product, and muscle enzymes: creatine kinase (CK) and aspartate aminotransferase (AST) were determined in serum samples. Venous blood samples for determination of selected blood parameters were collected, immediately post exercise, and 24 and 48 hours post exercise.*

*Significant changes in most of the biochemical and haematological parameters determined immediately after exercise reflect the normal physiological response of horses to a selected field exercise test. Most of these parameters returned to or close to the stall values within 48 hours. The concentration of plasma MDA increased immediately post exercise, though not significantly; however it increased significantly 24 hours post exercise and reached its highest value 48 hours post exercise. Thus exercise-induced oxidative stress is evidenced by increased lipid peroxidation. From the rapid decline of serum CK activity post exercise and the absence of significant correlations between MDA and serum muscle enzymes, we concluded that the selected field exercise test caused no permanent alteration in muscle cell integrity or muscle damage.*

*Key words: biochemical parameters, exercise, haematological parameters, lipid peroxidation, muscle cell integrity, standardbred horses*

## INTRODUCTION

The horse has a regulatory system that responds in a complex manner to stress, such as exercise (Coenen, 2005). Physical exercise can modify the animal's metabolism (Kedzierski *et al.*, 2009). Thus, exercise testing is very

important and should include a range of physiological measurements related to the level of fitness of the animal (Tateo *et al.*, 2008).

Exercise-induced physiological processes are reflected in changes in blood constituents. In addition to higher activities of creatine kinase (CK), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), increased red blood cell (RBC) and white blood cell (WBC) counts, increased haemoglobin concentration (Hgb) and haematocrit values (Hct), increased plasma total protein (TP), albumin, glucose, urea, creatinine, inorganic phosphate (iP), cholesterol and lactate have been found post exercise (Hodgson and Rose, 1994; Kingston, 2004a; McGowan, 2008). Some other plasma components, such as potassium (K) and calcium (Ca), were decreased (Balogh *et al.*, 2001). Changes in blood flow, increased cardiac output and stimulation of sweat production are the initial consequences of catecholamine liberation (Coenen, 2005). During exercise there is, under the influence of catecholamines, a contraction of the spleen that results in the release of erythrocytes and therefore increased oxygen transport capacity (Hodgson and Rose, 1994; Kingston, 2004a; Art and Lekeux, 2005; McGowan, 2008).

During physical exercise, oxygen flux to active skeletal muscles increases, which leads to enhanced production of reactive oxygen species (ROS). Strenuous physical exercise may induce oxidative stress (Kinnunen *et al.*, 2005). The horse has the unique ability to increase its oxygen uptake by a factor of 60 during heavy exercise (Art and Lekeux, 2005), which results in increased mitochondrial production of ROS (Ji, 1999; Art and Lekeux, 2005), thus inducing lipid peroxidation (Dekkers *et al.*, 1996; Chiaradia *et al.*, 1998) and exposing the horse to exercise-induced oxidative stress (Balogh *et al.*, 2001). Skeletal muscle may be subjected to a greater level of oxidative stress during exercise than the liver or heart, due to increased ROS production (Ji, 1999). The main target substrates for ROS are polyunsaturated fatty acids in the membrane phospholipids. The modification of which results in disorganization of the cell framework and function. One of the principal and best known end products of lipid peroxidation is malondialdehyde (MDA), which serves as a reliable and most commonly used marker of the overall level of lipid peroxidation and presence of oxidative stress (Del Rio *et al.*, 2005; Grotto *et al.*, 2009). Increased ROS production could favour membrane lipid peroxidation, thereby decreasing muscle cells' membrane integrity (Dekkers *et al.*, 1996; Kirschvink *et al.*, 2008), which could lead to tissue damage (Art and Lekeux, 2005) and muscle fatigue (Marlin *et al.*, 2002). In order to estimate the extent of such lipid peroxidation induced by exercise, the well-known and easily measured end products of lipid peroxidation, such as MDA, are determined (Urso and Clarkson, 2003; Sachdev and Davies, 2008). Several reports have already shown an association between oxidative stress and skeletal muscle damage in sport horses (Chiaradia *et al.*, 1998; Frankiewicz-JóŹko and Szarska, 2000; White *et al.*, 2001; Hargreaves *et al.*, 2002; Williams *et al.*, 2003). Despite the increasing number of papers on changes in blood parameters induced by exercise, the effects of field exercise on several blood parameters related to energy utilization, as well as exercise induced lipid peroxidation in standardbred horses are still not well established.

The aim of the present study, therefore, was to examine changes in plasma MDA concurrent to haematological and biochemical profiles for trained standardbred horses post exercise and to determine whether 24 hours/48 hours is sufficient for selected blood parameters to return to their stall values. Additionally, correlations between MDA and serum muscle enzymes, CK and AST, whose increased activities may be indicative of muscle membrane leakage induced by oxidative stress were studied.

## MATERIALS AND METHODS

### *Horses, their diet and exercise protocol*

Ten clinically healthy standardbred horses comprising 5 geldings, 1 stallion and 4 mares, all between the ages of 2 and 10 years ( $6.2 \pm 3.05$  years) and all in the phase of basic conditioning training and with a history of racing, were used in the study. All horses were subjected to a comprehensive clinical examination, which included a general impression of the horse and habitus, a general clinical examination (rectal temperature, pulse, breathing, mucous membranes, lymph nodes) and detailed heart and lung auscultation and evaluation. The horses were judged to be healthy, based upon normal history, clinical examination, and results of haematological and biochemical blood analyses.

All horses had been on a wash-out period for 6 weeks before exercise, fed a diet consisting of 4 kg Tradition Cavalor complete industrial feed (Cavalor, Belgium), 1 kg black oats and 8 kg hay per day. No oral supplements were administered before or during the study. Water was provided *ad libitum*. During 6 weeks of wash-out period horses were subjected to basic activity (paddock, jogging) and received no medication.

At the time of the exercise (in March 2010), which took place on the racetrack in Ljubljana (Slovenia), the outdoor temperature was 5°C with air pressure approximately 983 millibars and relative humidity approximately 72 %. Each horse had to complete 4 periods of 500 m trotting (at the following speeds: 1 min 30 s/km, 1 min 20 s/km, 1 min 25 s/km and 1 min 20 s/km). After each period, horses had 3 minutes of rest. Prior to exercise, a pre warming up of 15 minutes was completed, consisting of 10 minutes walking and 5 minutes jogging. The heart rate of each horse was measured pre ( $35.2 \pm 4.02$  bpm) and post ( $122.5 \pm 8.76$  bpm) exercise activity.

The study was approved by the Veterinary administration of the Republic of Slovenia.

### *Collection and preparation of blood samples*

Blood samples for the determination of plasma MDA and haematological and biochemical parameters, were collected from the jugular vein prior to exercise (in stall), within 5 minutes after exercise (post exercise), 24 hours after exercise (rest 1) and 48 hours after exercise (rest 2).

Blood samples for biochemical profile determination were collected in serum separator tubes (Vacuette; Greiner Bio-One, Kremsmunster, Austria) and left for 1h to clot prior to centrifugation at 1300 g for 10 minutes at 4°C. Serum was

separated and aliquoted into two cryotubes. Biochemical profiles were determined on the day of blood collection.

Blood samples for plasma glucose and lactate determination were collected in tubes containing lithium iodoacetate and heparin (Vacuette; Greiner Bio-One, Kremsmuenster, Austria). Tubes were centrifuged at 1500 g for 15 minutes at 4°C. Plasma was separated and analysed on the day of blood collection.

Tubes with K<sub>3</sub>EDTA anticoagulant (Vacuette; Greiner Bio-One, Kremsmunster, Austria) were used for collecting blood samples for the determination of complete blood count (CBC), white cell differential count (WCDC) and MDA. EDTA blood samples for determining CBC and WCDC were stored at room temperature and analysed between 2 and 3 hours after sampling.

Tubes for plasma MDA determinations were centrifuged at 1500 g for 15 minutes at 4°C. Plasma was separated and immediately frozen at -80°C until analysis.

#### *Biochemical analyses*

Biochemical profiles included electrolytes, sodium (Na), potassium (K), chloride (Cl), magnesium (Mg), inorganic phosphate (iP), calcium (Ca), total protein (TP), albumins (alb), CK, AST, alanine aminotrasferase (ALT), ALP, glucose, lactate, creatinine, urea, triglycerides (TG), cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Electrolyte concentrations were determined with an electrolyte analyser Ilyte Na/K/Cl (Instrumentation Laboratory, Lexington, MA, USA). Other biochemical parameters were determined with an automated biochemistry analyser RX-Daytona (Randox, Crumlin, UK).

#### *Determination of haematological parameters*

CBC and WCDC were determined with an automated laser haematology analyser Technicon H\*1 (Siemens, Munich, Germany) with species specific software (H\*1 Multi-Species V30 Software, Tarrytown, New York, USA). The resulting CBC includes white (WBC) and red (RBC) blood cell counts, haemoglobin concentration (Hgb) and haematocrit (Hct). WCDC comprises neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EOS), basophils (BASO) and large unstained cells (LUC), all as absolute values. The LUC category consists of a heterogeneous population of large cells that fail to exhibit any peroxidase activity (atypical lymphocytes, immature granulocytes and blasts).

#### *Determination of malondialdehyde*

Total MDA concentration in plasma samples was determined using a derivatization procedure with 2,4-dinitrophenylhydrazine and high-performance liquid chromatography (HPLC) according to Pilz *et al.* (2000) 250 µL of plasma samples and 50 µL of 6 M sodium hydroxide were mixed and incubated at 60°C for 30 minutes. The samples were then acidified with 125 µL of 5.25 M perchloric acid and centrifuged at 5°C and 15000 rpm for 10 minutes. 250 µL of supernatant was mixed with 25 µL of 5 mM 2,4-dinitrophenylhydrazine solution and incubated

at room temperature for 30 minutes. Derivatized samples were extracted twice with hexane, the organic phase evaporated under a nitrogen stream, reconstituted with 200  $\mu$ L of mobile phase, and 20  $\mu$ L injected into the HPLC system. HPLC analyses was performed on an Agilent 1100 series system (Waldbronn, Germany) equipped with a UV detector operating at 310 nm. The chromatographic separation was carried out with a Gemini C18 150  $\times$  4.6 mm column with 5  $\mu$ m particle size (Phenomenex, Torrance, CA). The column was kept at 35°C. Mobile phase flow-rate was 0.9 mL/min. The mobile phase consisted of 0.2% acetic acid in a 62:38 (v/v) water and acetonitrile mixture.

#### Statistical analysis

Data is shown as means  $\pm$  standard deviation of the means (SD).

In order to test differences of each parameter with time of sampling, One way repeated measures analysis of variance in the case of normal distribution and Friedman repeated measures analysis of variance on ranks when data were not normally distributed were used.  $p < 0.05$  was considered as significant. Spearman rank correlation analysis was used to determine the correlation between MDA and serum muscle enzymes, CK and AST.

SigmaStat 3.5 (SYSTAT Software Inc.) software was used for statistical evaluation of the results.

## RESULTS

During the study, horses were clinically healthy and all haematological and biochemical parameters measured in the stall were within the physiological range for standardbreds (Hodgson and Rose, 1994; Kingston, 2004b). There was a significant difference in plasma MDA at different times of sampling. Plasma MDA was higher post exercise than the stall value (Figure 1), however the difference

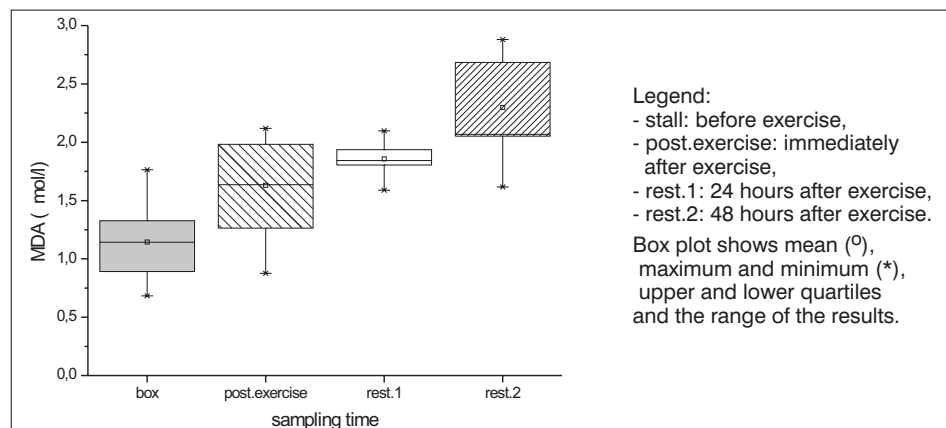


Figure 1. Plasma MDA concentrations of standardbred horses, determined before and after exercise

was not significant. Plasma MDA was significantly higher in the rest 1 and rest 2 samples than in the stall values (Figure 1). Correlations between MDA and CK and between MDA and AST values were not significant at any sampling time.

The activity of CK was significantly higher post exercise than in the stall samples. It decreased after resting; values at rest 1 and rest 2 were close to stall values (Figure 2).

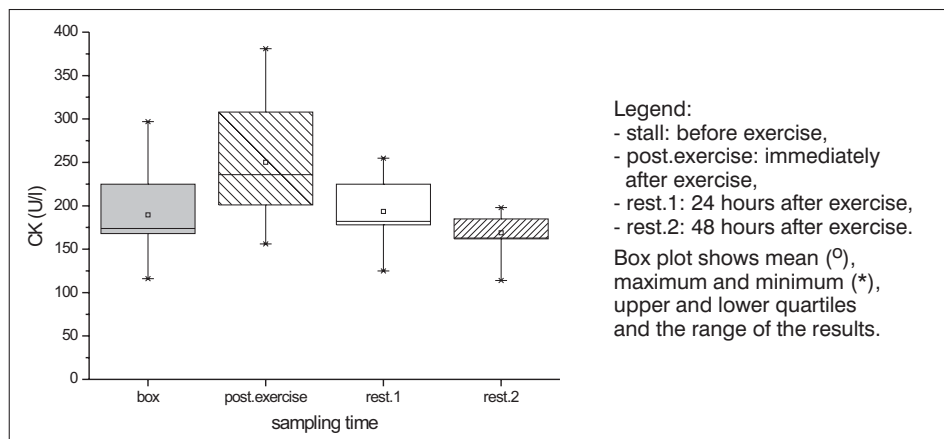


Figure 2. Serum creatine kinase (CK) activity of standardbred horses, determined before and after exercise

There were significant differences between AST levels at different times of sampling, however multiple pairwise comparisons revealed a significant difference only between post exercise and rest 2 (Figure 3).

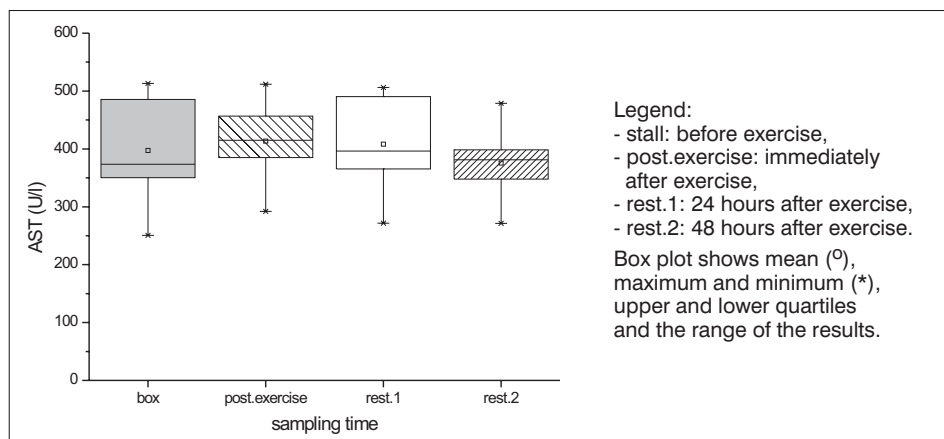


Figure 3. Serum aspartate aminotransferase (AST) activity of standardbred horses, determined before and after exercise

Plasma lactate concentration was significantly higher post exercise than at stall sampling time. After resting, the level of lactate decreased; values at rest 1 and rest 2 did not differ significantly from stall values (Figure 4).

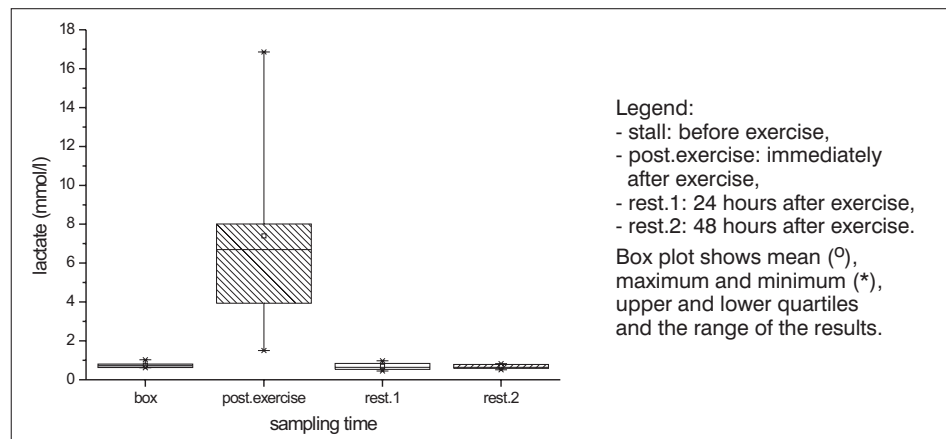


Figure 4. Plasma lactate concentrations of standardbred horses, determined before and after exercise

Haematological parameters are presented in Table 1. The values of RBC, Hgb, Hct, WBC and LUC were significantly higher post exercise than in stall samples, however, they returned to near stall values after resting. NEUT counts were significantly higher post exercise than stall values. Despite the fact that they were lower at rest 1, they were still significantly higher than at stall sampling time and returned to near stall values at rest 2. LYMPH counts decreased on resting; at rest 2, LYMPH counts were significantly lower than at rest 1 and post exercise, and did not differ from stall values. BASO, MONO and EOS values did not show any significant differences between sampling times.

Biochemical parameters are shown in Table 2. With the exception of glucose and HDL, all biochemical parameters changed significantly between sampling times.

At post exercise the levels of ALP, ALT, creatinine, alb, Na and Cl differed significantly from stall values; all returned to near stall values at rest 1, with the exception of ALP, whose values returned to stall value at rest 2. Post exercise values of Ca and TG differed significantly from stall values. Moreover, they remained different from stall values at rest 1 and rest 2. Urea increased from stall values at rest 1 and remained increased at rest 2. K and cholesterol decreased significantly between rest 2 and all other sampling times, while significant decreases in LDL were observed between stall and rest 1 and rest 2. The level of iP differed significantly from stall values only at rest 2. TP and Mg at all three sampling times post exercise did not differ from stall values, although a significant difference was observed between post exercise and rest 1 for Mg and between post exercise and both rest 1 and rest 2 for TP.



Table 1. Mean values ( $\pm$ SD) of haematological parameters determined in 10 standardbred horses at each sampling time

Measured parameters	Stall	Post exercise	Rest 1	Rest 2
RBC ( $\times 10^{12}/L$ )	8.89 $\pm$ 0.95 <sup>a</sup>	11.49 $\pm$ 0.68 <sup>b</sup>	9.01 $\pm$ 0.97 <sup>a</sup>	8.44 $\pm$ 0.72 <sup>a</sup>
Hgb (g/L)	141.80 $\pm$ 11.63 <sup>a</sup>	186.80 $\pm$ 12.25 <sup>b</sup>	142.70 $\pm$ 10.81 <sup>a</sup>	135.20 $\pm$ 9.52 <sup>a</sup>
Hct (L/L)	0.39 $\pm$ 0.03 <sup>a</sup>	0.51 $\pm$ 0.04 <sup>b</sup>	0.40 $\pm$ 0.03 <sup>a</sup>	0.37 $\pm$ 0.03 <sup>a</sup>
WBC ( $\times 10^9/L$ )	6.21 $\pm$ 1.43 <sup>bc</sup>	7.69 $\pm$ 1.92 <sup>a</sup>	6.79 $\pm$ 1.27 <sup>ac</sup>	5.95 $\pm$ 1.30 <sup>b</sup>
NEUT ( $\times 10^9/L$ )	2.93 $\pm$ 0.83 <sup>a</sup>	3.52 $\pm$ 0.90 <sup>b</sup>	3.47 $\pm$ 0.91 <sup>ab</sup>	3.10 $\pm$ 0.76 <sup>a</sup>
LYMP ( $\times 10^9/L$ )	2.57 $\pm$ 0.75 <sup>ab</sup>	3.38 $\pm$ 1.36 <sup>b</sup>	2.63 $\pm$ 0.52 <sup>b</sup>	2.19 $\pm$ 0.61 <sup>a</sup>
MONO ( $\times 10^9/L$ )	0.40 $\pm$ 0.14 <sup>a</sup>	0.37 $\pm$ 0.15 <sup>a</sup>	0.38 $\pm$ 0.09 <sup>a</sup>	0.37 $\pm$ 0.12 <sup>a</sup>
EOS ( $\times 10^9/L$ )	0.16 $\pm$ 0.10 <sup>a</sup>	0.13 $\pm$ 0.05 <sup>a</sup>	0.17 $\pm$ 0.11 <sup>a</sup>	0.16 $\pm$ 0.13 <sup>a</sup>
BASO ( $\times 10^9/L$ )	0.35 $\pm$ 0.01 <sup>a</sup>	0.07 $\pm$ 0.06 <sup>a</sup>	0.03 $\pm$ 0.01 <sup>a</sup>	0.03 $\pm$ 0.01 <sup>a</sup>
LUC ( $\times 10^9/L$ )	0.12 $\pm$ 0.03 <sup>a</sup>	0.19 $\pm$ 0.06 <sup>b</sup>	0.12 $\pm$ 0.04 <sup>a</sup>	0.10 $\pm$ 0.03 <sup>a</sup>

a,b,c,d Mean values with different superscripts in the same row differ significantly ( $p < 0.05$ )

Table 2. Mean values ( $\pm$ SD) of biochemical parameters determined in 10 standardbred horses at each sampling time

Measured parameters	Stall	Post exercise	Rest 1	Rest 2
ALP (U/L)	103.79 $\pm$ 24.60 <sup>a</sup>	115.85 $\pm$ 23.94 <sup>b</sup>	109.85 $\pm$ 23.81 <sup>ab</sup>	97.47 $\pm$ 16.43 <sup>a</sup>
ALT (U/L)	13.02 $\pm$ 3.40 <sup>b</sup>	16.23 $\pm$ 4.61 <sup>a</sup>	14.77 $\pm$ 3.67 <sup>b</sup>	13.49 $\pm$ 2.66 <sup>ab</sup>
Urea (mmol/L)	5.70 $\pm$ 0.60 <sup>b</sup>	5.50 $\pm$ 1.02 <sup>b</sup>	6.68 $\pm$ 0.55 <sup>a</sup>	6.35 $\pm$ 0.74 <sup>a</sup>
Creatinine ( $\mu$ mol/L)	111.05 $\pm$ 15.94 <sup>a</sup>	140.96 $\pm$ 9.92 <sup>b</sup>	115.10 $\pm$ 11.79 <sup>a</sup>	115.18 $\pm$ 11.80 <sup>a</sup>
TP (g/L)	61.63 $\pm$ 3.04 <sup>ab</sup>	65.57 $\pm$ 4.19 <sup>b</sup>	60.30 $\pm$ 3.09 <sup>a</sup>	60.32 $\pm$ 4.77 <sup>a</sup>
Alb (g/L)	34.87 $\pm$ 1.22 <sup>a</sup>	37.37 $\pm$ 1.78 <sup>b</sup>	34.96 $\pm$ 1.43 <sup>a</sup>	34.78 $\pm$ 1.84 <sup>a</sup>
Glucose (mmol/L)	4.23 $\pm$ 0.63 <sup>a</sup>	4.27 $\pm$ 1.69 <sup>a</sup>	4.65 $\pm$ 0.47 <sup>a</sup>	4.18 $\pm$ 0.57 <sup>a</sup>
Na (mmol/L)	136.57 $\pm$ 1.03 <sup>a</sup>	137.79 $\pm$ 1.32 <sup>bc</sup>	136.70 $\pm$ 1.78 <sup>ac</sup>	137.90 $\pm$ 1.26 <sup>b</sup>
K (mmol/L)	3.82 $\pm$ 0.35 <sup>a</sup>	3.57 $\pm$ 0.43 <sup>a</sup>	3.63 $\pm$ 0.40 <sup>a</sup>	3.12 $\pm$ 0.44 <sup>b</sup>
Cl (mmol/L)	99.78 $\pm$ 1.38 <sup>a</sup>	97.92 $\pm$ 2.20 <sup>b</sup>	98.69 $\pm$ 1.47 <sup>ab</sup>	99.42 $\pm$ 1.09 <sup>a</sup>
Ca (mmol/L)	3.17 $\pm$ 0.16 <sup>a</sup>	2.96 $\pm$ 0.16 <sup>b</sup>	2.94 $\pm$ 0.10 <sup>b</sup>	3.00 $\pm$ 0.17 <sup>ab</sup>
iP (mmol/L)	1.00 $\pm$ 0.23 <sup>a</sup>	1.12 $\pm$ 0.23 <sup>ab</sup>	1.16 $\pm$ 0.24 <sup>ab</sup>	1.26 $\pm$ 0.16 <sup>b</sup>
Mg (mmol/L)	0.77 $\pm$ 0.12 <sup>ab</sup>	0.70 $\pm$ 0.09 <sup>a</sup>	0.82 $\pm$ 0.12 <sup>b</sup>	0.77 $\pm$ 0.09 <sup>ab</sup>
TG (mmol/L)	0.31 $\pm$ 0.07 <sup>c</sup>	0.52 $\pm$ 0.19 <sup>ab</sup>	0.65 $\pm$ 0.16 <sup>a</sup>	0.50 $\pm$ 0.11 <sup>b</sup>
Cholesterol (mmol/L)	2.34 $\pm$ 0.43 <sup>a</sup>	2.53 $\pm$ 0.45 <sup>a</sup>	2.29 $\pm$ 0.38 <sup>a</sup>	2.01 $\pm$ 0.29 <sup>b</sup>
HDL (U/L)	1.20 $\pm$ 0.18 <sup>a</sup>	1.13 $\pm$ 0.16 <sup>a</sup>	1.08 $\pm$ 0.13 <sup>a</sup>	1.15 $\pm$ 0.16 <sup>a</sup>
LDL (U/L)	0.39 $\pm$ 0.09 <sup>a</sup>	0.39 $\pm$ 0.07 <sup>ab</sup>	0.37 $\pm$ 0.09 <sup>b</sup>	0.37 $\pm$ 0.10 <sup>b</sup>

a,b,c,d Mean values with different superscripts in the same row differ significantly ( $p < 0.05$ )



## DISCUSSION

The increased plasma MDA concentrations, and significant changes of most of the haematological and biochemical parameters, reflect the response of these horses to a selected field exercise test. Although significant differences were observed, the values of all the haematological and biochemical parameters remained within the physiological range for standardbreds (Hodgson and Rose, 1994; Kingston, 2004b) at all sampling times.

It has been suggested that modifications of cell membranes caused by lipid peroxidation following ROS overproduction may be one of the causes of exercise-induced myopathies and haemolysis in horses (Chiaradia *et al.*, 1998; Marlin *et al.*, 2002). In the present study, only a slight increase in plasma MDA concentration was observed immediately after exercise. Similar results have been observed in thoroughbred race horses in the racetrack post exercise (Kedzierski *et al.*, 2009), with no significant changes 30 minutes after rest (Kedzierski *et al.*, 2009). Contrary to our results, a study conducted on thoroughbred racehorses (White *et al.*, 2001) demonstrated significantly elevated MDA concentrations 5 minutes after a race. The methods for plasma MDA determination have, however, differed between studies.

After the post exercise sampling time, plasma MDA concentrations continued to increase, reaching their highest values in the next 48 hours. Thus, plasma MDA values were significantly higher 24 hours and 48 hours post exercise than stall values, indicating increased lipid peroxidation. Similarly, Avellini *et al.* (1999) reported significantly elevated plasma MDA concentrations in racehorses after the maximum power test, and Chiaradia *et al.* (1998) reported significantly elevated plasma MDA concentrations 18 hours after exercise in racehorses. Moreover, elevated plasma MDA concentrations have been found 14 days after an endurance race in racehorses (Al-Qudah and Al-Majali, 2008). The continuing rise in plasma MDA after exercise probably indicates the slow process of elimination of lipid peroxidation end products (Chiaradia *et al.*, 1998).

Since the activity of CK and AST returned to near basal values as soon as 24 hours after exercise, and as there were no significant correlations between plasma MDA and the CK and AST, it may be assumed that increased lipid peroxidation caused by the selected field exercise test did not cause significant skeletal muscle cell damage. Similar results have been reported in sport horses (Chiaradia *et al.*, 1998).

The increases in CK activity post exercise shown in the present study could cause a transient increase in muscle cell membrane permeability, thus showing a normal physiological response to exercise (Tateo *et al.*, 2008; Teixeira-Neto *et al.*, 2008) rather than any permanent alteration in cellular integrity (Hodgson and Rose, 1994). The moderate rises in plasma AST post exercise observed in the present study also suggest that no permanent alteration in muscle cell integrity took place (Snow *et al.*, 1982). Unlike muscle enzymes, only a few effects of intense or submaximal exercise are seen on liver enzymes (McGowan, 2008). Although, ALT and ALP activities increased significantly immediately after exercise, the increase was not clinically important (Chiaradia *et al.*, 1998).

Lactate is widely used to examine the effects of conditioning and diagnose positive performance (Lindner, 2000) and to assess the level of fitness in sport horses (Piccione *et al.*, 2010a). In the present study, significantly increased values of plasma lactate were found post exercise compared with those of stall values, in agreement with previous studies conducted on standardbreds (Piccione *et al.*, 2010a), Polo horses (Zobba *et al.*, 2011), endurance horses (Al-Qudah and Al-Majali, 2008) and other sport horses (Lindner, 2000). Prompt lactate recovery after exercise is an index of good animal fitness (Zobba *et al.*, 2011). Mean plasma lactate values in the present study decreased significantly as soon as 24 hours post exercise, showing good fitness of the standardbred horses.

In the present study RBC, WBC, Hct and Hgb increased significantly immediately after exercise. However their values returned to the stall values within 24 or 48 hours post exercise, in accordance with other studies conducted on sport horses (Piccione *et al.*, 2010b; Padalino *et al.*, 2007; Kingston, 2004a). The increase in RBC, Hct and Hgb can be attributed mainly to reflex splenic contractions, which occur in horses in response to fright, excitement, and exercise (Ricketts, 2004).

Increases in both urea and creatinine have been detected in most studies in response to high and low intensity exercise (Hodgson and Rose, 1994). A significant increase in creatinine was observed post exercise in the present study, probably indicating dehydration as a result of extensive fluid loss in the sweat (Piccione *et al.*, 2010b). Concentrations of urea were significantly higher 24 and 48 hours after exercise than in the stall values. The continuing rise of urea indicates that protein catabolism continues after the cessation of exercise (Snow *et al.*, 1982).

The changes of albumins, TP, sodium, chloride and calcium indicate dehydration due to physical activity (Nemec Svete *et al.*, 2008; Coenen, 2005). The concentration of iP in the present study remained significantly higher 48 hours post exercise than stall values, which could be due to the intensity of exercise, with no need for the high energy of ATP (Arslan *et al.*, 2002). The results of the present study show significantly lower potassium concentration 48 hours post exercise than at all other sampling times, which could be due to both loss in sweat and influx from contracting muscles (Snow *et al.*, 1983).

As observed previously in standardbred horses (Piccione *et al.*, 2010b; Tateo *et al.*, 2008), significantly higher TG levels post exercise have been demonstrated in the present study. Determination of plasma TG level has not been generally accepted for monitoring the post exercise changes in horses, although it is assumed that lipid metabolism in horses varies according to the intensity or duration of exercise, as well as to breed, age and sex (Kedzierski *et al.*, 2009). This fact could be related to the changes in cholesterol and LDL determined in the present study.

Different exercise intensities and modalities, fitness status of sport horses, sample collection timing, as well as analytical methodologies can contribute to the differences between studies, making it difficult to compare results across studies.

In the present study, the changes of haematological and biochemical parameters reflect a normal physiological response to a selected field exercise test in standardbred horses. Twenty-four or 48 hours of rest was enough for most of the haematological and biochemical parameters to return to their stall values after exercise. In contrast, plasma MDA continued to increase after exercise and reached its highest values 48 hours post exercise, which clearly indicates increased lipid peroxidation due to increased production of ROS during exercise. Given the rapid decline of CK after exercise and the absence of significant correlations between MDA and serum muscle enzymes CK and AST, we can conclude that exercise-induced oxidative stress as evidenced by increased lipid peroxidation did not cause any permanent alteration in muscle cell integrity. On the basis of results from the present study we also conclude that the standardbred horses were in good physical fitness.

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#### KONCENTRACIJA MALONIL-DIALDEHIDA U KRVNOJ PLAZMI, BIOHEMIJSKI I HEMATOLOŠKI PARAMETRI KOD AMERIČKOG KASAČA TOKOM ODABRANIH TESTOVA OPTEREĆENJA NA TERENU

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#### SADRŽAJ

Cilj ove studije je bio da se utvrde promene u koncentraciji malonil-dialdehida (MDA) u krvnoj plazmi kao i hematološki i biohemijski profil kod 10 klinički zdravih američkih kasača podvrgnutih odabranim testovima opterećenja na terenu. U krvnom serumu je određivan i odnos između koncentracije MDA, krajnjeg produkta glavne lipidne peroksidacije i aktivnosti enzima mišića, kreatin kinaze (CK) i aspartat aminotransferaze (AST). Uzorski venske krvi za analize su prikupljeni u mirovanju, neposredno po završetku opterećenja a zatim i 24 i 48 sati nakon toga.

Neposredno po završetku opterećenja, utvrđene su značajne promene većine biohemijskih i hematoloških parametara i one se mogu smatrati normalnim fiziološkim odgovorom. Većina ovih parametara se vratila na vrednosti registrovane u mirovanju u roku od 48 sati. Koncentracija MDA u krvnoj plazmi je odmah posle napora bila povećana, ali ovo povećanje nije bilo statistički značajno za razliku od vrednosti registrovanih 24 sata posle vežbe. Maksimalna vrednost MDA je registrovana nakon 48 sati. Ovaj oksidativni stres je bio indukovao opterećenjem što potvrđuje povećan stepen lipidne peroksidacije. Na osnovu brzog pada aktivnosti CK u serumu po završetku opterećenja i odsustvu značajne korelacije između koncentracije MDA i aktivnosti mišićnih enzima u serumu, može se zaključiti da odabrani testovi opterećenja nisu imali za posledicu trajne alteracije u mišićnim ćelijama i da nisu doveli do njihovog oštećenja.