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Original Scientific Article

CHANGES IN BLOOD ANTIOXIDANT, BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS IN POLICE HORSES ON DUTY

Peter Kruljc[1], Nina Čebulj-Kadunc[2], Robert Frangež[2], Alenka Nemec Svete[3]*

[1]Clinic for Reproduction and Horses, [2]Institute for Physiology, Pharmacology and Toxicology, [3]Clinic for Surgery and Small Animal Medicine, University of Ljubljana, Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia

*Corresponding author, E-mail: alenka.nemecsvete@vf.uni-lj.si

Summary: The objective of this study was to test the hypothesis that routine patrolling by police horses induces physiological changes of haematological and biochemical parameters, but not exercise-induced oxidative stress. Therefore, the activities of whole blood antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), activities of serum muscle enzymes, aspartate aminotransferase (AST) and creatine kinase (CK), as well as haematological and other biochemical parameters in police horses on duty were investigated. Correlations between antioxidant and muscle enzymes were also determined. Fourteen healthy, male, warm-blooded police horses were included in this study. Horses were transported to their place of duty where they patrolled for six hours at a walking pace, which was regarded as moderate physical activity. Blood samples for determination of SOD, GSH-Px, AST, CK, haematological and biochemical parameters were collected in the horses' stalls (basal values), after their transportation to the place of duty, immediately after duty, after transportation back to their stalls and after a 24-hour rest. No significant changes in the activities of antioxidant enzymes among different sampling times ocurred. Correlations between antioxidant and serum muscle enzymes activities were not significant at any of the sampling times. However, significantly higher ($p < 0.05$) AST and CK activities were found immediately after duty in comparison to basal values, which is probably the result of leakage from intact muscle fibres resulting from muscular activity, rather than a consequence of oxidative stress-induced muscle damage. Fluctuations of haematological and biochemical parameters reflect horses' physiological response to physical activity when on patrol. In conclusion, police horses did not develop exercise-induced oxidative stress while being subjected to moderate physical activity on duty. They appeared to be in good physical condition and fit for this type of duty. Moreover, our results indicate that the investigated horses could be used for patrolling for longer periods of time.

Key words: police horses; exercise-induced oxidative stress; antioxidant enzymes; serum muscle enzymes.

Introduction

Physical exercise has been shown to induce tissue damage via the oxidation of cellular components, such as membrane lipids, proteins and DNA (1,2). During exercise and rest, a number of potential intracellular sources of reactive oxygen species (ROS) generation exists in the skeletal muscles and heart. They are derived from the mitochondrial transport chain, xanthine oxidase catalysed reactions, NAD(P)H oxidase enzymes from the sarcoplasmic reticulum and plasma membrane redox systems. Superoxide anion is the main ROS in the muscle cell produced by either an incomplete reduction of oxygen in the electron transport chain or as a specific product of different enzymatic systems (3). Increased production of ROS may lead to exercise-induced oxidative stress (1,2,4). However, endogenous enzymatic and non-enzymatic antioxidants have demonstrated great adaptability in response to acute and chronic exercise (4,5).

Exercise-induced oxidative stress is believed to contribute to muscle fatigue and muscle fibre damage, which can lead to exercise intolerance

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and poor performance (5,6). Horses are unique athletes within the animal kingdom. Their survival in the wild depends partly on their capacity to provide explosive effort when necessary, in order to escape predators. Consequently, during heavy exercise a horse has a unique ability to almost instantaneously increase its oxygen uptake by factors of more than 60, which leads to increased mitochondrial production of ROS (1,4,7). The latter could favour membrane lipid peroxidation of muscle cells and thereby decrease their membrane integrity, which could lead to tissue damage and muscle enzyme leakage (1,8). Associations between exercise-induced oxidative stress and muscle enzyme leakage have already been confirmed in sport horses (9,10,11).

Prolonged low-medium intensity exercise has been reported to induce oxidative stress in humans (5,12). In exercising horses, numerous studies have shown that exercise-induced oxidant/antioxidant changes vary with regard to the type of exercise (racing, standardised treadmill exercises, standardised race track exercises, endurance) and markers assessed in blood, although it is generally agreed that physical exercise does induce significant alterations in the circulatory oxidant/antioxidant balance. However, some controversy exists in terms of poorly reproducible and even contradictory results, which suggests that experimental design, the horses' fitness, the analytical approach and environmental factors strongly influence the study results (8,9,13,14,15) Physical exercise not only induces oxidative stress but can also modify the animal's physiological metabolism, leading to changes of physiological, haematological and biochemical parameters (7,16,17,18,19,20). In horses, all the studies that have investigated the impact of physical exercise on the oxidant/antioxidant balance and physical, haematological and biochemical parameters were conducted in sport horses, but none in police horses, which are daily subjected to different kinds of physical activity when on duty. The objective of this study was to test the hypothesis that routine patrolling of police horses induces physiological changes of haematological and biochemical parameters, but not exercise-induce oxidative stress. Therefore, we determined the activities of whole blood antioxidants, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), activities of serum muscle enzymes, aspartate aminotransferase (AST) and creatine kinase (CK), as well as haematological and selected biochemical parameters in police horses on duty (patrolling). In addition, correlations between whole blood antioxidant and serum muscle enzymes, whose increased activities may indicate muscle cell leakage induced by exercise-induced oxidative stress, were determined.

Material and methods

Horses

The study was conducted on fourteen healthy warm-blooded police horses, geldings, which were from five to seventeen years old (10.88 ± 0.87 years; n=14). They were transported for 80 kilometres in a double-horse trailer, with an average speed of 40 km/h, to the place of duty where they patrolled for the next 6 hours (from 10 a.m. to 4 p.m.). While patrolling, horses were ridden at walk through hilly woods, which was regarded as moderate physical activity. Horses were resting from 12 p.m. to 12:30 p.m. when they were provided food and water. The height difference that police horses had to overcome while patrolling was 274 m and approximate distance travelled was 35 km. After patrolling, they were transported back to their stalls. The transportation took approximately 2 hours in one direction. The horses were given water *ad libitum* and fed three times on the day they were on duty. At 5:30 a.m. and after transportation back at their stalls horses were given hay, oats and Cavalor Essential briquettes (Cavalor, Drongen, Belgium), at 12:00 p.m. horses were given only hay. The horses investigated were on the same duty the week before the study presented was performed.

All the horses included in this study were healthy and in a normal physical condition throughout the study. They belonged to the same mounted police department and were, therefore, subjected to the same police training programme, diet, vaccination and anti-helmintic procedures. While off duty, investigated police horses were exercised daily according to the following official training programme, which was set by police horse trainer: gymnastics exercise in manège (1 hour/day; gallop, gait, trot, etc.) or conditional field work (one and a half hour/day). Horses were normally exercised six days per week; one day per week was a resting time. At least twice a month,

the investigated horses were subjected to indoor and outdoor police polygon training that included: dressage, resting, gymnastics exercise and horse controlling, resting and training with disturbing elements (vigorous noises, 'smoke' test, gun shoots, alarm and other loud sounds, etc.). Twice a year, the investigated horses were also subjected to police polygon training (outdoor) in durations of three and five days. The training included outdoor tracking, dressage, restraint training, training with disturbing elements and final exercises.

During the study, the horses were carefully monitored for signs of injury, such as muscular pain, stiffness or lameness.

Collection and preparation of blood samples

Blood samples for determination of whole blood antioxidant enzymes, haematological and biochemical parameters were collected by venepuncture of the left jugular vein in the stall prior to transportation (basal values), immediately (within 5 minutes) after transportation to the place of duty (postTr1), after duty (postD) and after transportation back to their stall (postTr2), as well as after a 24-hour rest (resting-stall).

Blood samples for the determination of serum cortisol and biochemical profile, with the exception of lactate and glucose, were collected into serum separator tubes (Vacuette; Greiner Bio-One, Kremsmunster, Austria) and were left still for 30 minutes to clot prior to centrifugation at 1300 g at 4°C for 10 minutes. The serum was then separated and aliquoted into two cryotubes. Serum samples for the determination of cortisol concentration were kept frozen at -20 ± 2°C until analysed within two months after sampling. Biochemical profiles were determined on the day of sampling.

Blood samples for plasma glucose and lactate determination were collected into 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 sampling.

Tubes with K_3EDTA anticoagulant (Vacuette; Greiner Bio-One, Kremsmunster, Austria) were used for the collection of blood samples for the determination of haematological parameters. Blood samples containing EDTA were stored at room temperature (20–22 °C) and analysed within 10 hours after sampling.

Blood samples for determining GSH-Px and SOD activities were collected into tubes containing the anticoagulant lithium heparin (Vacuette; Greiner Bio-One, Kremsmunster, Austria) and frozen within 10 hours after sampling at -80°C until analysis. The samples were analysed within two months after blood sample collection.

Biochemical analyses and serum cortisol determination

Biochemical profiles included serum electrolytes (sodium (Na), potassium (K), chloride (Cl), magnesium (Mg), inorganic phosphate (iP), calcium (Ca)), creatinine, urea, total protein, albumin, alkaline phosphatase (AP), CK, alanine aminotransferase (ALT) and AST, as well as plasma glucose and lactate. Na, K and Cl concentrations were determined with an electrolyte analyser Ilyte Na/K/Cl (Instrumentation Laboratory, Lexington, MA, USA). Biochemical profiles, with the exception of Na, K and Cl, were determined with an automated biochemical analyser RX-Daytona (Randox, Crumlin, UK).

Serum cortisol concentrations were determined using a commercial enzyme immunoassay (Active Cortisol EIA, DPC, Los Angeles, USA). Absorbances of calibrators and samples were measured spectrophotometrically using a microplate reader Anthos (Anthos Labtech Instruments GmbH, Salzburg, Austria). Cortisol concentrations were calculated by the WinRes computer programe, which is a functional part of the reading system. All samples were analysed with a single run, with variability coefficients of 2.05 and 5.29 for low ($\overline{X}$ =116.75 nmol/l) and high ($\overline{X}$ = 694.42 nmol/l) values, respectively.

Haematological analyses

Haematological analyses were performed using a Technicon H*1 automated laser haematology analyser (Siemens, Munich, Germany) with species-specific software (H*1 Multi-Species V30 Software, Tarrytown, New York, USA). The complete blood count (CBC) included white blood cells (WBC), red blood cells (RBC), haemoglobin concentration (Hgb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT). The white cell

differential count (WCDC) represent a six-part differential: neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EOS), basophils (BASO) and large unstained cells (LUC). WCDC values are represented as a percentage. The LUC category consists of a heterogeneous population of all large cells that fail to exhibit any peroxidase activity (atypical lymphocytes, immature granulocytes and blasts).

Measurement of GSH-Px activity

The activity of GSH-Px in whole blood haemolysates was determined spectrophotometrically with an automated biochemical analyser RX-Daytona (Randox Laboratories, Crumlin, UK) using the commercial Ransel kit (Randox Laboratories, Crumlin, UK), which is based on the method of Paglia and Valentine (21). The activity of GSH-Px was expressed as Units/g of haemoglobin (U/g Hgb).

Measurement of SOD activity

SOD activity in whole blood haemolysates was determined spectrophotometrically with an automated biochemical analyser RX Daytona (Randox Laboratories, Crumlin, UK), using a commercially available Ransod kit (Randox Laboratories, Crumlin, UK), which is based on the original method of McCord and Fridovich (22). Activity was expressed as U/g Hgb.

Statistical evaluation

Data was analysed using the SPSS computer program (SPSS 17.0 for Windows, Chicago, Illinois, USA). Results are expressed as means ± standard error of the mean (s.e.m.). Changes of measured parameters (significant difference) were assessed with repeated measures of ANOVA with the Bonferroni correction. Pearson's correlation coefficient analysis was performed to determine the correlation between the activities of whole blood antioxidants and serum muscle enzymes at each sampling time. A value of $p < 0.05$ was considered significant.

Results

No significant changes in the activity of antioxidant enzymes were observed among different sampling times, while the activity of serum muscle enzymes, AST and CK, changed significantly (Table 1). In comparison to basal values, the activity of AST was significantly higher at postTr1, postD and postTr2; while the activity of CK was significantly higher at postD sampling time. However, the activities of AST and CK returned to near their basal values after a 24-hour rest.

Whole blood antioxidants and serum muscle enzymes activities were not significantly correlated at any sampling times (data not shown).

Table 1: Whole blood antioxidant and serum muscle enzymes activities (mean ± s.e.m.) in police horses before and after duty

Measured parameter	Reference ranges	Basal values	PostTr1	PostD	PostTr2	Resting-stall
SOD (U/g Hgb)	1411.6±246.9[23] 1284.0±81.0[24]	1220.3±65.0[a]	1247.8±62.8[a]	1252.0±59.0[a]	1223.2±66.6[a]	1237.2±63.4[a]
GSH-Px (U/g Hgb)	215.0±9.0[24]	177.4±6.7[a]	175.9±5.5[a]	177.1±5.1[a]	180.2±8.0[a]	177.4±6.3[a]
AST (U/L)	160-412[24]	263.4±10.0[a]	276.9±12.3[b]	287.2±10.4[b]	303.3±13.2[c]	273.5±22.4[abc]
CK (U/L)	60-330[24]	165.5±15.5[a]	200.3±22.4[ab]	223.7±21.3a[b]	226.7±32.5[b]	199.0±21.4[ab]

[a,b,c]mean values with different superscripts in the same row differ significantly ($p < 0.05$); SOD - superoxide dismutase; GSH-Px - glutathione peroxidase; AST - aspartate aminotransferase; CK - creatine kinase

Table 2: Serum cortisol and biochemical parameters (mean ± s.e.m.) in police horses before and after duty

Measured Parameters	Reference ranges	Basal values	PostTr1	PostD	PostTr2	Resting-stall
Cortisol (µg/dL)	3.0-13.0[25]	18.7±4.1[a]	20.6±4.0[a]	20.5±4.2[a]	16.6 ±4.3[ab]	10.1±3.2[b]
Lactate (mmol/L)	<1.0[26]	0.65±0.04	0.65±0.05	0.81±0.09	0.74±0.05	0.66 ±0.05
Urea (mmol/L)	3.9-9.6[25]	5.3± 0.2[a]	5.4±0.2[abc]	5.6±0.2[abc]	5.8±0.2[b]	5.0±0.2[ac]
Creatinine (µmol/L)	35.4-194.5[25]	133.3±4.2[ab]	133.6±2.8[ab]	141.9±4.2[a]	141.1±4.6[ab]	125.6±4.2[b]
Glucose (mmol/L)	3.44-7.43[25]	5.8±0.2	5.4±0.1	6.0±0.2	5.8±0.1	5.5±0.1
Total protein (g/L)	56-76[25]	66.9±1.3[a]	68.8±1.2[ab]	69.6±0.8[bc]	72.1±1.4[c]	69.3±1.1[bc]
Albumin (g/L)	26-41[25]	39.1±0.6[a]	40.1±0.5[abd]	41.4±0.5[bd]	43.3±0.7[c]	41.3±0.4[cd]
Na (mmol/L)	128-142[25]	139.5±0.5[acd]	137.8±0.4[bd]	139.3±0.7[d]	140.8±0.9[c]	138.8±0.4[bcd]
K (mmol/L)	2.9-4.6[25]	3.60±0.08[a]	4.99±0.21[b]	4.62±0.13[bd]	4.14±0.24[acd]	3.29±0.17[ca]
Cl (mmol/L)	98-109[25]	99.6±0.5[a]	99.6±0.4[ab]	100.4±0.6[ab]	101.7±0.6[b]	99.3±0.4[ab]
Ca (mmol/l)	2.55-3.35[25]	3.01±0.07	2.99±0.07	3.03±0.07	3.26±0.26	3.00±0.08
Mg (mmol/L)	0.58-0.94[25]	0.88±0.03[ab]	0.87±0.03[ab]	0.83±0.03[a]	0.83±0.03[ab]	0.91±0.03[b]
iP (mmol/L)	0.48-1.52[25]	0.94±0.07[ab]	0.98±0.07[ab]	0.83±0.04[a]	0.97±0.07[ab]	1.11±0.05[b]
AP (U/L)	102-2[57]27	108.5±7.2[ab]	107.1±6.6[a]	114.8±6.2[ab]	117.5±7.7[b]	118.1±8.8[ab]
ALT (U/L)	3.0-23.0[28]	18.2±1.4	17.9±0.9	20.2±1.0	20.1±0.8	19.8±1.6

[a,b,c,d]mean values with different superscripts in the same row differ significantly ($p < 0.05$); Na - sodium; K - potassium; Cl - chloride; Ca - calcium; Mg - magnesium; iP - inorganic phosphate; AP - alkaline phosphatase; ALT - alanine transferase

Table 3: Haematological parameters (mean ± s.e.m.) in police horses before and after duty

Measured parameters	Reference range[A]	Basal values	PostTr1	PostD	PostTr2	Resting-stall
WBC (x10^9/L)	5.40-12.00	6.39±0.35[a]	7.30±0.39[b]	7.83±0.44[b]	7.74±0.38[b]	7.00±0.32[b]
RBC (x10^{12}/L)	6.80-12.90	7.64±0.18[a]	8.08±0.13[ab]	8.41±0.21[ab]	8.18±0.18[ab]	8.13±0.14[b]
HCT (L/L)	0.32-0.53	0.35±0.01	0.38±0.01	0.39±0.01	0.38±0.01	0.37±0.01
Hgb (g/L)	110-180	136.8±4.0	143.9±2.1	149.6±2.8	145.4±2.1	142.2±2.3
MCV (fL)	34.0-55.0	46.0±0.8[a]	47.0±0.8[b]	46.9±0.8[b]	47.0±0.8[b]	46.4±0.7[ab]
MCH (pg)	12.3-19.0	17.8±0.3	17.8±0.2	25.2±7.2	17.8±0.3	17.8±0.3
MCHC (g/L)	340.0-410.0	387.9±4.0[a]	379.7±3.8[b]	381.1±4.1[b]	379.7±4.2[b]	384.9±2.9[ab]
PLT (x10^9)	50-350	145.8±7.9	138.9±5.6	142.7±4.9	145.6±7.1	144.9±7.4
NEUT (%)	40-75	58.0±1.6[ae]	65.1±1.6[b]	69.5±1.4[c]	71.7±1.1[cd]	59.4±2.2[abe]
LYMPH (%)	20-50	33.4±1.5[ae]	26.4±1.2[b]	24.5±1.0[bc]	22.6±0.9[d]	32.9±1.9[e]
MONO (%)	2-8	2.8±0.6	1.9±0.4	1.5±0.4	1.0±0.2	1.7±0.4
EOS (%)	0-5	1.3±0.3[ac]	0.9±0.3[ac]	0.5±0.3[b]	0.6±0.3[bd]	1.4±0.4[abd]
BASO (%)	0-2	0.30±0.02	0.40±0.08	0.30±0.04	0.30±0.06	0.30±0.03
LUC (%)	0-2	4.3±0.6	5.3±1.0	3.7±0.4	4.0±0.3	4.5±0.4

[a,b,c,d]mean values with different superscripts in the same row differ significantly ($p < 0.05$); [A]Reference ranges for horses (data from haematological analyser Technicon H*1); WBC - white blood cells; RBC - red blood cells; Hgb - haemoglobin concentration; HCT - haematocrit; MCV - mean corpuscular volume, MCH - mean corpuscular haemoglobin; MCHC - mean corpuscular haemoglobin concentration; PLT - platelets; NEUT - neutrophils; LYMPH - lymphocytes; MONO - monocytes; EOS - eosinophils; BASO - basophils; LUC - large unstained cells (atypical lymphocytes, immature granulocytes and blasts)

Serum cortisol and the biochemical parameters are presented in Table 2. With the exception of glucose, Ca, ALT and lactate, all other biochemical parameters changed significantly among different sampling times.

The concentration of serum cortisol did not change significantly immediately after duty or after both transports. However, the mean value was significantly lower at resting-stall than at basal, postTr1 and postD sampling times.

Compared to its basal value, a significantly higher urea concentration was determinated at postTr2, but it decreased significantly after resting, reaching the basal value. The concentration of creatinine was higher at postD and postTr2 than at basal value, however the differences were not significant. After a 24-hour rest, the concentration of creatinine returned to near basal concentration. Concentrations of total serum protein and albumin were significantly higher at postD, postTr2 and resting-stall sampling times than at basal values.

No significant changes of Na and Cl concentrations were observed immediately after duty when compared to their basal values. However, the concentration of Na was significantly lower at postTr1 and the concentration of Cl significantly higher at postTr2 sampling time in comparison to respective basal values. There were some significant changes in the concentration of these two electrolytes during other sampling times. Potassium concentrations were significantly higher postTr1 and postD in comparison to basal concentration. After resting, Na, K and Cl concentrations were near their respective basal values. Concentrations of Mg and iP did not change significantly at any sampling times when compared to basal values, but their concentrations increased significantly from postD to resting-stall sampling time.

The activity of AP did not change significantly at any sampling times when compared to its basal value. However, the activity was significantly higher at postTr2 than at postTr1 sampling time.

Some of the haematological parameters, WBC, RBC, MCV, MCHC, NEUT, LYMPH and EOS changed significantlly among different sampling times (Table 3). In comparison to their basal values, significantly higher WBC values were determinated at postTr1, postD, postTr2 and resting-stall sampling times, and significantly higher RBC values at resting-stall sampling time. MCV and MCHC values were significantly higher postTr1, postD and postTr2 than their basal values.

Compared to basal values, significantly higher NEUT values and significantly lower LYMPH values were determinated at postTr1, postD and postTr2 sampling times. After a 24-hour rest, the values of NEUT and LYMPH returned to near basal values. Significantly lower EOS values were determinated at postD and postTr2 than at basal measurements.

Discussion

Determination of the changes of whole blood antioxidant and serum muscle enzyme activities, as well as haematological and biochemical parameters, provides valuable information about the physiological adjustments of police horses to stressful conditions that these horses experienced while being on duty.

At the beginning of the study, the basal values of all haematological and biochemical parameters were measured. With the exception of the serum cortisol concentration that slightly exceeded the upper value of its reference range (it will be discussed later), all parameters were within their reference ranges (25,26,27,28).

Antioxidant enzymes, SOD and GSH-Px, constitute the primary antioxidant defence system against ROS and are thus sensitive markers of oxidative stress (5,29). An association between exercise-induced oxidative stress and muscle enzyme leakage has already been confirmed in sport horses (9,10,11), but not in police horses on duty. The present study established no significant correlations between whole blood antioxidant and serum muscle enzyme activities. Significantly higher activities of serum AST and CK found in investigated horses immediately after duty in comparison to their basal values are probably the result of leakage from intact muscle fibres resulting from muscular activity (19), rather than a consequence of oxidative stress-induced muscle damage. The latter is also supported by moderate increases in AST and CK activities immediately after duty and a decline after a 24-hour rest. It has been suggested that sampling at least 24 hours after exercise may reveal the differences between those animals showing a normal physiological response to exercise and those with abnormal or pathological response (19). In comparison to its

basal value, AST activity increased significantly after both transports. The transport of animals is physically demanding and can result in muscle damage and consequently in an increased activity of serum muscle enzymes (30). Despite significant increases of CK and AST activities, the values remained within their reference ranges (25).

Growing evidence indicates that the antioxidant defence systems of mammalian tissues are capable of adaptation in response to acute and chronic exercise (4,5). However, in sport horses, significantly higher (9,10,13,15) or significantly lower activity (31) of blood antioxidant enzymes, either SOD and/or GSH-Px, have been reported post-exercise as a consequence of enhanced ROS production, either by up-regulation of enzyme activity or utilisation of the antioxidant enzymes to counter the ROS (4,5). In contrast, the results of some studies demonstrate no significant changes of antioxidant enzyme activities post-exercise in comparison with pre-exercise values (18,31). The inconsistency of results on blood SOD and GSH-Px activities could be a reflection of differences in exercise intensity, duration, type, or training (8). The present study demonstrated no significant changes of SOD and GSH-Px activities in police horses on duty. The lack of significant changes in antioxidant enzymes might be ascribed to the low intensity and short duration of physical activity of police horses on duty, as well as to the acclimatisation of investigated horses to this kind of physical activity since they have been on the same route many times and are daily subjected to exercise training programme. Activities of antioxidant enzymes determined in investigated horses were in general agreement with previously reported data at all sampling times (9,23,24).

During exercise, both the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis are activated to improve oxygen delivery to the working muscles. The activation of these systems is demonstrated by rapid increases in the circulating levels of adrenocorticotropin (ACTH), cortisol, adrenaline and noradenaline, allowing these hormones to be utilised in the evaluation of exercise-induced stress (32,33). Increased concentrations of cortisol in plasma or serum were reported only in sport horses after physical exercise (17,20,32,34,35). In the present study, the concentration of serum cortisol exceeded the upper value of the reference range (25) from the beginning of the study (basal value) to postTr2 sampling time, with resting-stall values being within the reference range. Higher than normal concentrations of serum cortisol were determined in investigated horses, which could have been due to the pre-duty excitement that horses experience in their stalls before being transported to the place of duty and later due to transport stress (36). The level of serum cortisol did not change significantly immediately after duty in comparison to basal values, but its concentration was significantly lower after a 24-hour rest than at all other sampling times. This result might be ascribed to the effect of training (37) since police horses are trained regularly on the same route.

As a response to exercise, the level of lactate found in blood is generally regarded as an indicator of fitness and degree of training, because it reflects the dependence on anaerobic metabolic pathways (33,38,39). Significantly increased concentrations of plasma lactate have been reported post-exercise in sport horses (16,20,34,40). In the present study, plasma lactate remained within the reference range (26) at all sampling times and did not differ significantly among sampling times. The lack of plasma lactate response to the physical activity of investigated horses is probably a consequence of the training (7,41) of these horses, and the fact that the anaerobic metabolism was not reached in these horses due to moderate type of physical activity. The results of lactate concentrations indicate that the police horses used in this study were in good physical condition.

Patrolling of police horses resulted in a significant increase of total serum protein, albumin and potassium concentrations at postD sampling time. Significantly increased albumin and total serum protein concentrations, determined at postD, as well as at postTr2 and resting-stall sampling times, most probably indicate dehydration (25,42). A significant increase in serum potassium concentration at postTr1 and postD sampling times might be ascribed to the movement of potassium from muscle cells during muscular contraction, which is reflected by an increase in blood potassium concentration (42). It has been noted that the degree of exercise needed to produce this effect is slight (20 to 50 m of walking) and that continued light exercise does not result in further increase in plasma potassium (17).

Exercise has variable effects on the haemogram depending on work intensity (19). In human beings and most other animal species, exercise

results in physiologic leukocytosis associated with mobilisation of marginated neutrophils to the circulating pool and moderate to marked neutrophilia. In horses, the mobilisation of leukocytes based on excitement and exercise is masked by the concomitant increase in erythrocytes and blood volume as a result of splenic contraction (19,33). In the present study, the WBC count was significantly higher, not only at postD, but also at all other sampling times when compared to basal values. However, leukocytosis was not determined at any of sampling times. Concomitant with significant increases in the leukocyte count, relative values of neutrophils were significantly higher and relative values of lymphocytes significantly lower at postD, postTr1 and postTr2 than at basal sampling time, which could be attributed to the mobilisation of marginated neutrophils (19,33). The alterations in the differential count in the present study were those of a typical stress pattern and similar to those reported by Snow et al. (1982) in horses during prolonged exercise (17). Despite significant changes, relative values of neutrophils and lymphocytes remained within their reference ranges (Technicon H*1).

Exercise generally results in the mobilisation of splenic erythrocytes and, therefore, increases the oxygen transport capacity. The extent of the haematocrit increase is a function of exercise intensity. However, it should be noted that part of this increase is attributable to exercise-induced fluid shifts from intravascular to interstitial space (19,33). Significant increases in RBC, haematocrit and HGB were reported after different kinds of exercise in sport horses (16,17,20). In the present study, RBC were significantly higher at resting-stall in comparison to basal values, although the highest mean values were determined immediately after duty. These results might be due to the high spread of RBC measurements at postD sampling time, thus resulting in the occurrence of a high standard error. In association with the increases in RBC were elevations of haematocrit and haemoglobin values. The highest values of these two parameters were determined immediately after duty. However, the increase was not significant at any of the sampling times when compared to basal values, which might be due to moderate intensity of physical activity of police horses on duty.

Dehydration or haemoconcentration are features of exercise and heat exhaustion in horses performing in hot dry climates and over long distances (typically endurance races) (43). Total serum protein and albumin concentrations, as well as haematocrit, are often used as indicators of dehydration (25,42,44). In the present study, significantly increased serum total protein and albumin concentrations, as well as increases in RBC and haematocrit, determined at sampling times after the basal measurements indicate the development of a progressive dehydration.

In conclusion, the police horses did not develop exercise-induced oxidative stress while being subjected to moderate physical activity on duty. Fluctuations of haematological and biochemical parameters reflect horses' physiological response to physical activity when on patrol. After a 24-hour rest most of the measured parameters return to their basal values. On the basis of our results, we can also conclude that police horses used in this study were in good physical condition and fit for this type of duty. Moreover, our results indicate that the investigated horses could be used for patrolling for longer periods of time.

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SPREMEMBE KRVNIH ANTIOKSIDANTNIH, HEMATOLOŠKIH IN BIOKEMIJSKIH PARAMETROV POLICIJSKIH KONJ PRI NJIHOVEM SLUŽBENEM DELU

P. Kruljc, N. Čebulj-Kadunc, R. Frangež, A. Nemec Svete

Povzetek: Namen raziskave je bil testirati hipotezo, da rutinsko patruljiranje policijskih konj povzroči fiziološke spremembe hematoloških in biokemijskih parametrov, ne pa oksidativnega stresa kot posledice zmerne fizične aktivnosti, kateri so bili konji podvrženi med patruljiranjem. S tem namenom smo v raziskavi ugotavljali aktivnosti antioksidantnih encimov, superoksidne dismutaze (SOD) in glutationske peroksidaze (GSH-Px) v polni krvi, mišičnih encimov, aspartat-aminotransferaze (AST) in kreatin-kinaze (CK) v serumu kakor tudi vrednosti hematoloških in ostalih biokemijskih parametrov pri policijskih konjih med njihovim službenim deluom. Hkrati smo določali razmerja med antioksidantnimi in mišičnimi encimi. V raziskavo smo vključili 14 zdravih toplokrvnih policijskih konj. Odpeljani so bili na mesto, od koder so nato pričeli 6-urno patruljiranje s hodom. Med patruljiranjem so bili podvrženi zmerni fizični aktivnosti. Vzorce za določanje SOD, GSH-Px, AST in CK ter hematoloških in biokemijskih parametrov smo odvzeli v boksih domačega hleva (bazalne vrednosti), po njihovem transportu na mestu opravljanja službenega dela, takoj po opravljanem delu, po transportu nazaj v bokse ter po 24-urnem počitku. Med različnimi odvzemi krvi nismo ugotovili značilnih sprememb v aktivnosti antioksidantnih encimov. Ugotovili pa smo, v primerjavi z bazalnimi vrednostmi, značilno ($p < 0,05$) višje aktivnosti AST in CK takoj po opravljenem delu, kar je verjetno prej posledica prepuščanja mišičnih encimov iz nepoškodovanih mišičnih vlaken in mišične aktivnosti kot pa oksidativnega stresa. Nihanja hematoloških in biokemijskih parametrov odražajo normalen fiziološki odgovor na zmerno fizično aktivnost policijskih konj pri svojem službenem delu. Ugotovimo lahko, da zmerna fizična aktivnost, kateri so bili podvrženi policijski konji med rutinskim patruljiranjem ni povzročila oksidativnega stresa in da so konji v dobri fizični pripravljenosti ter primerni za tovrstno službeno delo. Poleg tega naši rezultati kažejo, da bi lahko policijske konje, ki so bili vključeni v raziskavo, uporabili za daljši čas patruljiranja.

Ključne besede: policijski konji; oksidativni stres kot posledica vadbe; antioksidantni encimi; serumski mišični encimi