Comparative Clinical Pathology (2023) 32:461–466
https://doi.org/10.1007/s00580-023-03457-2

ORIGINAL ARTICLE

Oxidative stress biomarkers status in selected equine sports

Ahmed S. Zohier[1] · Taher A. Baraka[2] · Ahmed Ismael Abdelgalil[3] · Amal M. Aboelmaaty[4] · Shimaa G. Yehia[2]

Received: 23 December 2022 / Accepted: 9 February 2023 / Published online: 23 February 2023

Abstract

Oxidative stress is an imbalance between oxidants and antioxidants. Chronic exercise can lead to an increase in the production of oxidants and the up-regulation of antioxidant defense mechanisms. In horses, there are several potential exercise-related mechanisms for oxidant production. Athletic horses are exposed to different amounts of stress according to the type of sport and physical exertion. The present study aimed to investigate the effect of type of sport, gender, and age on oxidant/antioxidant and enzymatic status in active athletic horses. Thirty-nine healthy athletic show-jumping, polo, tent-pegging, and normal work horses were evaluated for superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), total antioxidant capacity (TAC), lactate dehydrogenase (LDH), creatine kinase (CK), and alkaline phosphatase (ALP) at rest. A significant positive correlation between SOD and TAC was observed. CK was significantly higher in show-jumping horses compared to tent-pegging horses and higher in mares compared to stallions. ALP was significantly higher in polo horses compared to show-jumping horses. LDH was significantly higher in horses aged 11–14 compared to the 7–10-year group. Our findings support the occurrence of the adaptive response of oxidative/antioxidant status because of regular training in different equestrian sports disciplines, and stallions, mares, and geldings show similar adaptations to exercise-induced oxidative stress. Show-jumping seemed to cause more muscle damage than tent-pegging, especially in mares. A good physical condition may be observed in horses aged between 11 and 14 years.

Keywords Oxidative stress · Horse · Show jumping · Polo · Tent-pegging

Introduction

Oxidative stress has been recently linked to a variety of systemic disorders (Yehia et al. 2020). It occurs because of an imbalance between antioxidants and oxidants. Antioxidant defense mechanisms can be divided into enzymatic and non-enzymatic groups. An enzymatic group, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). Nonenzymatic ones such as vitamins C and E. Antioxidants are essential in disabling harmful metal ions and their role in producing free radicals (Martinovic et al. 2009; Hadžović-Džuvo et al. 2014). Free radicals and reactive oxygen species (ROS) are produced as part of cellular metabolic processes. The harmful effects of free radicals are caused by the need to maintain electronic stability, so they react with the next stable molecule, taking its electron and forming a new free radical (Hadžović-Džuvo et al. 2014). Although the harmful effects of ROS, low levels of them are necessary for important physiological functions such as cell signaling, apoptosis, and immune response (Hadžović-Džuvo et al. 2014; Shankar et al. 2014).

✉ Shimaa G. Yehia
drshimaa_ghanem@cu.edu.eg

Ahmed S. Zohier
drahmedzohier@gmail.com

Taher A. Baraka
drtaherbaraka@yahoo.com

Ahmed Ismael Abdelgalil
ismael7591@cu.edu.eg

Amal M. Aboelmaaty
amalaboelmaaty@gmail.com

1 Horse Training Department, Ministry of Interior, Cairo, Egypt

2 Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

3 Department of Surgery, Anesthesiology, and Radiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

4 Veterinary Research Institute, National Research Centre, Cairo, Egypt

Chronic exercise causes an increase in the production of oxidants, which leads to the up-regulation of antioxidant defense mechanisms (SOD, CAT, and GPx) in humans (Hadžović-Džuvo et al. 2014; D'Angelo and Rosa 2020). SOD and superoxide anion were the best indicators between volleyball player groups with different training experiences (Martinovic et al. 2009).

In horses, exercise has been shown to cause oxidative stress, which induces muscle damage and higher levels of lipid peroxidation (Kent et al. 2022). Several potential mechanisms exist for exercise-related oxidant production (Deaton and Marlin 2003). Horses have a special ability among sports animals to enhance their oxygen uptake by a factor of 60 during intense activity, which may boost ROS release during mitochondrial electron transfer. Regular training in equine athletes was found to stimulate the antioxidant defense mechanism (Andriichuk et al. 2016). There are possible pathways thought to be related to exercise-induced oxidative stress. (1) Inflammatory cell activation due to exercise-related injury and the subsequent production of ROS. (2) Exercise-induced hyperthermia causes an increase in lipid peroxidation in plasma and glutathione oxidation in red blood cell hemolysate. (3) The formation of superoxide due to anaerobic respiration of the hypoxic muscle fiber during stressful exercise (Deaton and Marlin 2003).

Trained horses had a significantly higher antioxidant barrier than untrained horses. As a result, frequent training helps horses adapt to larger amounts of oxidative substances during exercise, making them more resistant to oxidative stress and influencing oxidant/antioxidant balance (Fazio et al. 2016).

Creatinine kinase (CK) is a characteristic enzyme of muscular disorders that catalyzes the transfer of phosphate from phosphocreatine to ADP. Lactate dehydrogenase (LDH) is another enzyme found in muscle tissue that catalyzes the reversible conversion of pyruvate to lactate during glycolysis (Janicki et al. 2013). The intensity and duration of exercise are important factors in the onset of physiological fatigue. Muscle tissue may be damaged as a result of both metabolic and mechanical factors following intense prolonged training. As a result, this damage increases the permeability of the muscle membrane, which can result in the release of CK and all of the muscle damage biomarkers. (Mami et al. 2019).

Different anatomical structures could be exposed to a different amount of stress according to the type of sport and the amount and type of physical exertion (Murray et al. 2006; Bertuglia et al. 2014). The present study aimed to investigate the effect of type of sport, gender, and age on oxidant/antioxidant and enzymatic status in active athletic horses and to address the usefulness of these biomarkers in monitoring the health status of athletic horses.

Material and methods

Ethical statement

This study was conducted on horses belonging to a private governmental sector and was approved by the Institutional Animal Care and Use Committee (IACUC) of Veterinary Medicine, Cairo University, Certificate number Vet cut 20092022462.

Animal selection

Thirty-nine apparently healthy athletic horses (12 stallions, 14 geldings, and 13 nonpregnant mares) in the period between January 2020 and January 2021 were evaluated for oxidative stress biomarkers and enzymatic activities at rest. All clinical data (including animal description, management history, previous disease history, and treatment strategies) were collected.

The inclusion criteria included horses with an average age of 7–20 years that played (7) show-jumping, (7) polo, (15) tent-pegging, and (10) normal work for at least more than 3 years, and all horses were kept in the same equestrian club, stable, and trained on the same track.

All horses were housed in the same stable in individual boxes (4.00 × 4.00 m). The diet consisted of barely, soybean meal (SBM), wheat bran, alfalfa hay, bean husks, molasses, limestone (CaCo3), and salt, which was administered three times daily (at 7 AM, 1 PM, and 7 PM). Feed was analyzed by Regional Center for Food & Feed (RCFF), Giza Governorate, Egypt. The composition and the type of offered diet adapted to provide crude protein 12.6% DM, crude fiber 22.6% DM, and fats 4.39% DM. Water was available ad libitum.

All horses were receiving moxidectin PO (Equest®). Administer gel by inserting the syringe applicator into the animal's mouth depositing the gel in the back of the mouth near the base of the tongue. Once the syringe is removed, the horse's head is raised to insure proper swallowing of the gel.

Horses were assembled according to age into 4 groups, 7–10 years (7 horses), 11–14 years (9 horses), 15–17 years (13 horses), and > 18 years (10 horses) to estimate the differences in enzymatic activities and oxidative stress biomarkers between age groups.

Blood sampling and biochemical analysis

One blood sample was collected from each animal. A single examiner performed the blood sampling of 39 active athletic horses during the study period. Blood was collected from a jugular vein via vein puncture. Blood samples were obtained

Table 1 Oxidative stress biomarkers and enzymatic activities in different kinds of sports

Type of sport	*N*	SOD U/mL	CAT U/L	MDA nmol/mL	TAC mM/L	LDH U/L	CK U/L	ALP U/L
Tent-pegging	15	331.37 ± 22.64	79.29 ± 21.93	10.01 ± 0.01	4.99 ± 0.61	26.89 ± 5.28	26.08 ± 7.11[a]	13.9 ± 2.60
Polo	7	335.08 ± 32.68	80.45 ± 22.10	10.02 ± 0.01	4.36 ± 0.32	27.32 ± 4.49	35.77 ± 5.42	17.15 ± 3.28[b]
Show-jumping	7	417.02 ± 31.68	80.23 ± 14.67	9.99 ± 0.01	4.50 ± 0.40	24.96 ± 5.06	49.52 ± 6.29[a]	5.02 ± 1.20[b]
Normal work	10	379.41 ± 33.66	115.53 ± 21.86	10.01 ± 0.01	5.03 ± 0.18	19.83 ± 3.93	41.04 ± 7.45	12.26 ± 3.58

Values are means ± S.E.M

SOD superoxide dismutase, *CAT* catalase, *MDA* malondialdehyde, *TAC* total antioxidant capacity, *LDH* lactate dehydrogenase, CK, creatinine kinase, ALP, alkaline phosphatase

Means followed by the same superscript letters in columns differ significantly (Post hoc: Duncan's test at $P < 0.05$

for serum separation in plain tubes and on heparin anticoagulant for plasma and erythrocyte lysate preparation, which was obtained via centrifugation at 4000 rpm for 10 min, and the plasma was collected. The cells were rinsed three times with four volumes of cold saline, and the resulting red cell pellets were lysed by adding four volumes of cold deionized water to the estimated pellet volume. Centrifugation at 4000 rpm for 10 min at 4 °C was used to remove the red cell stroma. The resulting clarified supernatant was utilized to evaluate SOD using colorimetric test kits (Bio-Diagnostic Company, Egypt) according to the manufacturer's instructions (Paglia and Valentine 1967; Nishikimi et al. 1972), respectively. Samples were assayed on the same day. Serum was used to estimate serum parameters TAC and ALP using commercial colorimetric kits (Bio-Diagnostic, Egypt), CK, and LDH using enzymatic kinetic specified test kits using Bio-Diagnostic (Egypt) and Salucea (Italy), respectively.

CAT and MDA levels in heparinized plasma samples were determined using corresponding test kits (Bio-Diagnostic Company, Egypt) and made according to the manufacturer's instructions (Aebi 1984; Begenik et al. 2013).

Statistical analysis

Data are subjected to analysis using SPSS (version 25.0, 2016). Simple one-way ANOVA was used to study the effect of kind of sport, age, and gender on oxidative stress and blood biochemical parameters. Duncan's multiple range test was used to differentiate between significant means at $P < 0.05$. Pearson's correlation coefficient was used to correlate variables with each other.

Results

There was a significant positive correlation between SOD and TAC ($r = 0.36$; $P = 0.\ 0.026$).

Oxidative stress biomarkers and enzymatic activities in different kinds of sports are shown in Table 1. Mean CK concentration was significantly higher in show-jumping compared to tent-pegging horses. Mean ALP concentration was significantly higher in polo horses compared to show-jumping horses. There was no significant difference in mean SOD, CAT, MDA, TAC, and LDH between show-jumping, polo, tent-pegging, and normal work horses.

Oxidative stress biomarkers and enzymatic activities in different genders are shown in Table 2. Mean CK concentration was significantly higher in mares compared to stallions. There was no significant difference in mean SOD, CAT, MDA, TAC, ALP, and LDH between males, females, and geldings.

Oxidative stress biomarkers and enzymatic activities in different age groups are shown in Table 3. Mean LDH concentration was significantly higher in group 11–14-year horses compared to 7–10-year horses. There was no significant difference

Table 2 Oxidative stress biomarkers and enzymatic activities in mares, stallions, and geldings

Gender	*N*	SOD U/mL	CAT U/L	MDA nmol/mL	TAC mM/L	LDH U/L	CK U/L	ALP U/L
Mares	13	355.77 ± 20.61	108.47 ± 18.59	10.01 ± 0.01	4.40 ± 0.28	16.59 ± 3.38	46.15 ± 6.75[a]	15.14 ± 3.65
Stallions	12	332.11 ± 34.19	63.41 ± 23.03	10.00 ± 0.01	4.96 ± 0.57	25.97 ± 4.94	26.60 ± 7.20[a]	13.21 ± 2.49
Gelding	14	387.08 ± 23.37	89.39 ± 15.44	10.01 ± 0.01	5.03 ± 0.46	29.68 ± 4.41	36.19 ± 5.07	9.32 ± 1.86

Values are means ± S.E.M

SOD superoxide dismutase, *CAT* catalase, *MDA* malondialdehyde, *TAC* total antioxidant capacity, *LDH* lactate dehydrogenase, *CK* creatinine kinase, *ALP* alkaline phosphatase

Means followed by the same superscript letters in columns differ significantly (Post hoc: Duncan's test at $P < 0.05$)

Table 3 Oxidative stress biomarkers and enzymatic activities in different age groups

Age	*N*	SOD U/mL	CAT U/L	MDA nmol/mL	TAC mM/L	LDH U/L	CK U/L	ALP U/L
7–10 y	7	372.90 ± 35.87	112.75 ± 24.64	10.01 ± 0.01	4.45 ± 0.43	16.87 ± 5.16[a]	36.85 ± 8.36	13.79 ± 4.21
11–14 y	9	354.58 ± 31.95	72.05 ± 22.33	10.02 ± 0.01	5.39 ± 0.93	33.39 ± 8.23[a]	33.02 ± 8.06	13.68 ± 2.49
15–17 y	13	329.75 ± 19.82	98.97 ± 25.54	10.00 ± 0.01	4.82 ± 0.2	21.34 ± 2.68	34.87 ± 7.05	13.67 ± 3.50
> 18 y	10	394.12 ± 37.42	75.17 ± 15.55	10.00 ± 0.01	4.49 ± 0.41	25.63 ± 4.27	42.19 ± 7.57	8.05 ± 1.75

Values are means ± S.E.M

SOD superoxide dismutase, *CAT* catalase, *MDA* malondialdehyde, *TAC* total antioxidant capacity, *LDH* lactate dehydrogenase, *CK* creatinine kinase, *ALP* alkaline phosphatase

Means followed by the same superscript letters in columns differ significantly (Post hoc: Duncan's test at $P < 0.05$)

in means SOD, CAT, MDA, TAC, CK, and ALP between different age groups.

Discussion

Different studies investigated the differences in oxidative stress parameters in athletic horses before and after exercise (Andriichuk et al. 2016; Fazio et al. 2016; Ono et al. 1990; Williams et al. 2005; Kirschvink et al. 2006). The impact of exercise on oxidative/antioxidant balance is conflicting in the previous literature. In our study, we evaluated the oxidative stress status in equine athletes involved in different kinds of equestrian disciplines, including tent-pegging, polo, and show-jumping.

The correlation results between the serum enzymatic activities and oxidative stress biomarkers revealed a significant positive correlation between SOD and TAC in the current study. In parallel with our findings, positive correlations between TAC and the activities of all antioxidant substances were observed (Fazio et al. 2016). TAC is the sum of the total antioxidant capacity of the nonspecific polo of antioxidants, which includes nonspecific antioxidants, metal chelators, and antioxidant enzymes (GPX, CAT, and SOD) (Finsterer 2012).

Also, our findings revealed that there was no significant difference in mean SOD, CAT, MDA, and TAC between horse sports groups. In line with our findings, no change in SOD or CAT activity pre- and post-exercise was also observed in thoroughbred racehorses (Ono et al. 1990). In previous studies, glutathione peroxidase and total glutathione levels decreased in endurance horses after an 80 km (Hargreaves et al. 2002) and a 140 km race (Marlin et al. 2002). Plasma MDA levels in Marwari horses increased immediately after exercise and then significantly decreased after 10 days, indicating the activation of the antioxidant defense mechanism due to moderate exercise (Dedar et al. 2017). Contradicting our findings, a significant increase in SOD and CAT activity and also a decrease in lipid peroxidation activity (MDA) due to regular exercise have been previously observed (Andriichuk et al. 2016; Fazio et al. 2016). The conflicting findings in different previous reports may be explained by the variation in breeds, gender, type of exercise, and amount of training load (Andriichuk et al. 2016; Kirschvink et al. 2006). So, our findings support the occurrence of the adaptive response of oxidative/antioxidant status due to regular training in different equestrian sports disciplines (Andriichuk et al. 2014). The oxidative stress-induced adaptation process of exercise is based on reduced oxidative stress as a result of improved antioxidant defenses, decreased basal oxidant generation, and reduced radical leak during oxidative phosphorylation (Andriichuk et al. 2014).

There was no significant difference in mean SOD, CAT, MDA, and TAC between different sex groups. Similarly, it has been shown that males and females show similar exercise-induced adaptations in antioxidant defenses at the same absolute workload (Andriichuk et al. 2014; Pepe et al. 2009). Also, no difference between men and women in SOD and CAT activities at different running distances over time was reported due to a nonsignificant gender–time interaction (Pepe et al. 2009). There were no gender-based differences in lipid peroxidation or muscle damage levels caused by vigorous exercise (Kaikkonen et al. 2002). On the contrary, total SOD and CAT activity were significantly higher in physically active male rats than in physically active female rats (Yamamoto et al. 2002). Our findings support that stallions, mares, and geldings show similar adaptations to exercise-induced oxidative stress over time.

Concerning the effect of sport type, gender, and age on enzymatic activities in this study, LDH concentration was significantly higher in group 11–14-year horses compared to 7–10-year horses but remained within the reference range. LDH levels in horses at rest gradually decreased as the animals' training condition advanced, and a relatively low increase in LDH occurred in more hard exercises if the animals were in good physical condition (Santos et al. 2015). Contrary to our results, LDH did not show significant age-related changes in Arabian mares, Bulgarian, and Murinsulaner horses (Gurgoze and Icen 2010; Prvanović Babić et al. 2019; Popova et al. 2020).

Higher CK activity in show-jumping horses compared to tent-pegging horses and significantly higher CK in mares compared to stallions was observed. All CK results are within the reference values previously reported by Knottenbelt and Malalana (2014). Physical exercise has been proven to produce physiological responses to meet increased metabolic demands during activities (Andriichuk et al. 2014). Show-jumping training with obstacles of 1 m triggered a metabolic change in CK (Carvalho et al. 2019). In the same line, significant increases in CK and LDH levels were observed in athletic mares after exercise, and no changes were detected in stallions (Filippo et al. 2016). Higher CK activity in mares may be attributed to more muscle damage than in stallions. The type, intensity, and duration of exercise all influence the profile of increased CK activity in the serum (Janicki et al. 2013). CK activity could be part of a pre-race evaluation of the horse's suitability to compete in that race (Williams et al. 2005).

ALP concentration was significantly higher in polo horses compared to show jumpers in the present study. ALP levels were found to be lower than the previously reported reference range (Knottenbelt and Malalana 2014; Satué et al. 2022). Different studies reported that ALP decreases with age (Gurgoze and Icen 2010; Muñoz et al. 2012). There was no significant difference in ALP results between the exercising and resting groups or pre- and post-exercise samples in Marwari horses (Dedar et al. 2017). The clinical significance of low serum ALP levels is unknown, so practitioners may underestimate them (Riancho-Zarrabeitia et al. 2016). Low ALP activity could be caused by vitamin C, zinc, and magnesium deficiency (Schmidt et al. 2021). Zinc and magnesium are necessary for adequate ALP activity in serum; so, low levels of these essential minerals may cause a reduction in ALP (Nanji 1982).

Conclusions

Our findings support the occurrence of the adaptive response of oxidative/antioxidant status due to regular training in different equestrian sports disciplines, and stallions, mares, and geldings show similar adaptations to exercise-induced oxidative stress over time. Show-jumping seemed to cause more muscle damage than tent-pegging, especially in mares. A good physical condition may be observed in horses aged between 11 and 14 years. A further understanding of the usefulness of oxidative stress biomarkers for monitoring the health status of athletic horses still needs more investigations on a larger number of athletic horses with different types of sports, genders, and age ranges.

Author contribution All authors contributed to the conception, design, and drafting of the study.

Data accessibility The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Compliance with ethical standards

Funding This study was not supported by any funding.

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval This study was conducted on horses belonging to a private governmental sector. This study was approved by Institutional Animal Care and Use Committee (IACUC) of Veterinary Medicine, Cairo University, Certificate number Vet cut 20092022462.

Informed consent For this type of study informed consent is not required.

Consent for publication For this type of study consent for publication is not required.

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Limitations The study was conducted on a small number of horses. Further studies are needed on a larger number.