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Short Communication

Relationship of Some Oxidative Stress Biomarkers in Jumper Horses After Regular Training Program

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ABSTRACT

In the present study, the redox status of blood samples, by assessing the total oxidant status (TOS), the total antioxidant capacity (TAC), and the oxidative stress index (OSI), together with the nitric oxide radical ($NO^{\bullet}$) metabolite (NOx) content, was evaluated in trained horses. Moreover, the oxidative damage to lipids and proteins, analyzed as thiobarbituric acid reactive substances (TBARS) and advanced oxidation protein products (AOPP), was also assessed. Twenty-five regularly trained Italian Saddle horses (7–10 year old, 12 geldings and 13 females, mean body weight 480 ± 30 kg) were enrolled in this study. Animals were carried on with their specific training programs for jumpers for 6 days per week with a day of rest, during which blood sampling was performed. On obtained serum samples, TOS, TAC, NOx, TBARS, and AOPP were measured. Our results showed significant linear correlations among markers of redox potential (TAC, TOS, and OSI) and among TOS and NOx with serum markers of lipid and protein oxidative injury (TBARS and AOPP). Furthermore, multiple linear regression analysis has shown that lipid peroxidation and protein oxidation levels are related to the content of oxidant substances (TOS and NOx). The obtained data can be useful to assess the status of an athletic horse and its adaptability to physical effort, providing an opportunity to modify the training schedule to achieve the desired performance.

1. Introduction

Oxidative stress (OS) depicts an imbalance between production of reactive oxygen species (ROS) and the capacity to detoxify reactive intermediates or to repair the consequent damage by an appropriate antioxidant defense leads [1]. OS causes damage of all the cellular components, such as proteins, lipids, carbohydrates, and nucleic acids [2].

In clinical methodology, different methods for OS evaluation are used, in which the biological samples to be analyzed are usually represented by plasma or serum. For routine analyses in clinical examination, the main characteristic of the applied methodologies is the ability to allow a rapid and not expensive data collection, capable of describing with reproducibility the oxidative status of animals.

In the first instance, the assessment of the balance between oxidants and antioxidants, known as redox potential, allows to know any situations in which oxidants may prevail for their accumulation or for an excessive consumption of endogenous and exogenous molecules being part of the antioxidant barrier.

Among proposed methods describing the total oxidant status (TOS) and the total antioxidant capacity (TAC) of

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serum or plasma, reactive oxygen metabolites (ROMs), and ferric reducing antioxidant power (FRAP) assays are commonly applied in equine medicine [3]. Furthermore, the analysis of the metabolites of nitric oxide radical ($NO^{\bullet}$), namely nitrite (NO_2^-), and nitrate (NO_3^-) or their sum (NOx) as indicated by Miranda [4] allows to indirectly assess the powerful oxidizing agent known as peroxynitrite (ONOOH), generated by reaction between $NO^{\bullet}$ and a variety of radicals and substances [5]. Regarding biomarkers to assess lipid and protein damage induced by free radicals in plasma or serum, thiobarbituric acid reactive substances (TBARS) and advanced oxidation protein products (AOPPs) assays are the commonly applied methods also in equine medicine to show OS occurrence [6,7].

In equine medicine, OS is recognized to be involved in many diseases and as consequence of athletic exercise [8–10]. Horse is a good model to study OS because its oxygen uptake increases 60 times above the basal value during maximal exercise [11], inducing serious generation of ROS in the mitochondrial electron transport system. Several researches have shown exercise-induced oxidant and/or antioxidant changes in trained horses. In particular, the effect of exercise on redox balance is extremely complex, depending on gender [12] and on intensity and duration of exercise [9,13–15]. Although regular moderate training appears beneficial for OS reduction and health [16], ROS overproduction can be induced by acute and strenuous bouts of aerobic and anaerobic exercise. Marin et al [17] showed that in most intense periods of training and competitions, a significant change of plasma OS parameters occurs. However, the chronic exposure to regular training exercise seems to improve antioxidant defense systems [16].

The aim of this study was to evaluate some OS biomarkers in regularly trained show jumper horses, in first instance through the evaluation of redox balance (TOS and TAC), together with $NO^{\bullet}$ metabolites (NOx). In addition, biomarkers of lipid and protein oxidation, as TBARS and AOPP, were also assessed to verify any possible relation among the applied methodologies, and between lipid and protein oxidation products and the oxidant contents, represented by TOC and NOx. This allows to estimate the lipid and protein damage by knowing the amounts of blood oxidants in regularly trained horses.

2. Materials and Methods

2.1. Experimental Procedure

Twenty-five regularly trained Italian Saddle horses (7–10 year old, 12 geldings and 13 females, mean body weight 480 ± 30 kg) from the same training center located in Sicily were investigated.

Before starting the study, horses have been subjected to clinical examination at rest conditions to verify their healthy status. All animals were housed in the same stable in individual boxes (3.50 × 3.50 m) under natural spring photoperiod (sunrise at 06:00 hours, sunset at 18:00 hours) and 18–21°C indoor temperature. The diet, consisted of hay (first cut meadow hay, sun cured, late cut; 8 ± 1 kg/day; 6.9% crude protein on average) and mixed cereals (oats and barley, 50% each, 3.5 ± 0.5 kg/day), was administered three times daily (at 7 AM, 12 PM, and 6 PM). Cereal mixture composition (dry matter basis) was 13.0% crude protein, 20.7% crude fiber, and 3.4% other extracts; the estimated net energy content was 0.8 UFC (Unitè Fouragire Cheval). Water was available *ad libitum*. Horses were carried on with their specific training programs for jumpers by the same trainer for 6 days a week with a day of rest, as indicated in our previous paper [18].

All treatments, housing, and animal care reported above were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU regarding the protection of animals used for scientific purposes, as recognized and adopted by the Italian law (DL 2014/26).

The horses were sampled on the same day at 8.00 AM before food administration; blood samples were collected from all subjects by jugular venipuncture using vacutainer tubes without anticoagulant agent. Blood samples were centrifuged at 2,500 g for 10 minutes at 4°C and the obtained the sera were stored at −80°C until analysis. On serum samples, TOS, TAC, NOx, TBARS, and AOPP were estimated.

2.2. Analytical Methods

Total oxidant status (TOS), measured as ROMs, was evaluated as described by Alberti et al [19] and was expressed as *tert*-butyl hydroperoxide (*t*-BHP) equivalents (μM). TAC was evaluated performing the FRAP assay [20]. Experimental data are presented as iron sulfate heptahydrate ($FeSO_4 \bullet 7H_2O$) equivalents (μM). Moreover, TOS/TAC ratio was accepted as oxidative stress index (OSI), as indicator of redox balance. Nitric oxide radical ($NO^{\bullet}$) was measured quantifying its stable metabolites (NOx), namely the sum of nitrite (NO_2^-) and nitrate (NO_3^-), as indicated by Miranda et al [4]. Experimental data are expressed as sodium nitrate ($NaNO_3$) equivalents (μM).

Thiobarbituric acid reactive substances (TBARSs) and advanced oxidation protein products (AOPPs) were evaluated as markers of lipid peroxidation and protein oxidation.

The concentration of AOPP was estimated according to Hanasand et al [21] and was expressed as of chloramine-T equivalents (μM). TBARSs were measured according to the method described by Ermis et al [22] and were expressed in terms of malondialdehyde (MDA) equivalents (μM) using an external standard calibration curve obtained from MDA generation with 1,1,3,3-tetraethoxypropane hydrolysis.

2.3. Statistical Analysis

Analytical data, presented as mean ± standard deviation (SD), are the averages of three analyses carried out by the same operator. Samples exhibited parallel displacement to the standard curve. The overall intra-assay coefficient of variation was <9%.

Basic descriptive statistics, including the measures of central tendency and dispersion, were calculated in according to Fanouraki et al [23].

A *t* test unpaired data were applied to evaluate statistically significant existing differences between geldings and females ($P < .05$).