Exercise-Induced Changes on Lipid Peroxides and Antioxidant Enzymes Levels Changes in Plasma of Show Jumping and Dressage Horses

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ABSTRACT
The objective of this study was to evaluate changes in oxidant and antioxidant parameters in plasma of horses competing in both jumping and dressage contests. In this study, 57 horses regularly involved in competition were included. Blood samples were taken at 3 time points: baseline at rest, upon reaching the schooling area but before exercise, and post-performance over a jump or dressage course. Fourteen healthy horses were considered as controls. Exercise induced an increase in lipid hydroperoxide (LPO) concentration in the 2 levels of horse jumping-show. We also observed an exercise-induced increase of LPO in dressage horse, although the effect was less apparent. By contrary, exercise decreased glutathione, glutathione peroxidase, and glutathione transferase levels. In conclusion, exercise-induced oxidative stress is linked with increased lipid peroxidation.

INTRODUCTION
Exercise is known to exert numerous physiolog-
ical changes in vital organ system of the body. Among those changes, the most important is the enhanced respiration and utilization of oxygen in the body. Recently, much evidence has accumulating indicating that exhaustive exercise generates free radicals. Abnormal production of free radicals leads to damage of some macromolecules, including proteins, lipids, and nucleic acids, and this is believed to be involved in the etiology of many diseases. Under normal conditions, excessive formation of free radicals and concomitant damage at cellular and tissue concentrations is controlled by cellular defense systems. These preventive defense systems can be accomplished by enzymatic or non-enzymatic mechanisms, including vitamin E, vitamin C, and glutathione. The antioxidant enzymes such as glutathione peroxidase (GPx) and glutathione-S-transferase (GST) may also have an important function in mitigating the toxic effects of reactive oxygen species.

Tissue glutathione plays a central role in antioxidant defense. Reduced glutathione detoxifies reactive oxygen species, such as hydrogen peroxide, and lipid peroxides directly or in a GPx-catalyzed mechanism. Glutathione also regenerates the major aqueous and lipid phase antioxidants, ascorbate and α-tocopherol. Glutathione-S-transferase catalyzes the reaction between the -SH group and potential alkylating agents, rendering them more water soluble and suitable for transport out of the cell. Glutathione transferase can also use peroxides as a substrate.

Although the exact mechanism for the exercise-induced cell and tissue damage is still elusive, there is increasing evidence that during strenuous exercise, the oxidation rate increases about 30-fold in horses. Thus, the exercising horse is a likely model to observe the balance between pro-oxidant assault and antioxidant defense.

In this study, we evaluated changes in oxidant and antioxidant parameters in plasma of horses competing in both jumping and dressage contests.

**MATERIALS AND METHODS**

Fifty seven horses between the ages of 5 to 12 years were sampled from 3 levels of horse show experience: moderate experience, intermediate experience: and most experimented. Fourteen healthy horses not involved in competition were used as control group.

Blood samples were collected from the jugular vein of each horse into EDTA tubes at 3 time points: baseline at rest, upon reaching the schooling but before exercise, and post-performance over a jump or dressage course. After sampling, plasma was separated and stored at -80°C until determinations were performed. Lactate concentration and lactate dehydrogenase (LDH) activity were measured spectrophotometrically.

Activities of GPx and GST, and lipid hydroperoxide (LPO) levels were determined using commercially available kits (Cayman Chemical Company, Ann Arbor, Michigan, USA).

Reproducibility within the assays was evaluated in 3 independent experiments. Each assay was carried out with 3 replicates. The overall intra-assay coefficient of variation has been calculated to be <5%. Assay-to-assay reproducibility was evaluated in 3 independent experiments. The overall inter-assay coefficient of variation has been calculated to be <6%.

**Statistical Analysis**

Results are expressed as the mean ± SEM. Mean comparison was done by the Kuskal-Wallis test followed by a Mann Whitney test; a confidence level of 95% (P < 0.05) was considered significant.

**RESULTS**

Baseline LPO (Figure 1A) and GPx plasma activity (Figure 1B) from both jumping and dressage horses did not differ from control horses, while both jumping and dressage horses show lower GST plasma levels than the control group (Figure 1C).

Competition induced a significant decrease in GPx levels in plasma at the 3
levels of horse jumping-show studied: moderate, intermediate, and most experienced horses (Figure 2). The exercise-induced effect was more apparent in dressage horses (Figure 3).

Plasma GST activity followed a pattern similar to that of GPx. After competition, GST activity was reduced in sport horses compared with baseline and before competition levels, both in jumping (Figure 4) and dressage (Figure 5) horses.

As shown in Figure 4, competition induced a dramatically decrease of GST levels in jumping horses with moderate experience. Although less marked, the
exercise-induced GST decrease was also observed in horses with intermediate experience, and most experienced horses. In dressage horses, this competition effect was even more apparent (Figure 5).

The decrease in antioxidant enzymes was accompanied by a competition-induced significant increase in LPO concentration in plasma at the 3 levels of horse jumping-show studied: moderate, intermediate, and most experienced horses (Figure 6). We also observed a competition-induced increase of LPO in dressage horses, although the effect was less apparent (Figure 7).
Competition also increases plasma concentrations of total protein, lactate, and LDH activity (data not shown).

DISCUSSION
There is growing evidence to support the theory that increased oxygen consumption during exercise increases free radical generation creating oxidative stress.\cite{2,7,12-16} Free radicals may play important roles within the body. They can act as chemical messengers and are also produced by phagocytes during the respiratory burst. Free radical formation can also increase under condi-
tions of altered metabolism, ischemia-reperfusion, and exercise.

Free radicals and oxidative stress have been implicated in the pathogenesis of different diseases, including exercise-induced cells and tissue damage. The role of reactive oxygen species (ROS) in the exercise process is supported by many studies. Production of ROS has been found to increase with exercise, thus augmenting the amount of oxidative damage induced to lipids, proteins, and DNA. This in turn may lead to dysfunction and inflammation.

In horses, oxidative stress has been shown to occur in some horses as a result of endurance exercise, in racehorses over several months of training, and as a result of exercise in several stressful environmental conditions. In the present study, we investigated the effect of jumping and dressage competition on free radical generation in sport horses.

Lipid peroxidation may be due to the oxidation of molecular oxygen to produce superoxide radicals. This reaction is also the source of $\text{H}_2\text{O}_2$, initiating the peroxidation of unsaturated fatty acids in the membrane. Both $\text{H}_2\text{O}_2$ and $\text{O}_2^-$ produced highly reactive hydroxyl radical with Haber-Weiss reaction. The hydroxyl radical can initiate lipid peroxidation, which is a free radical chain leading to loss of membrane structure and function.

In this study, the rate of lipid peroxidation, measured in the form of lipid peroxides, increased in the plasma after competition, suggesting exercise-induced oxidant stress. This is also supported by the simultaneous decrease in antioxidant enzymes, GPx and GST, which is in accordance with earlier reports of other authors. Some works, however, have shown either an increase or no change in antioxidant enzymes activity after exercise.

The aim of this study was to examine the effect of competition, and the control horses involved in the study were well-trained horses not participating in competitions.

In healthy individuals, there is balance between free radical generation and removal by antioxidants. The antioxidant defense is made up of both enzymatic and non-enzymatic parts, and changes in antioxidant enzyme activities in erythrocytes have been
used to document oxidative stress; between them, GPx and GST have been widely used.

An increase in oxygen consumption during exercise activates the enzyme GPx to remove hydrogen peroxide and organic hydroperoxides from the cell. Glutathione peroxidase is located in both the mitochondria and the cytosol where it serves as an important cellular protectant against free radical-induced damage to membrane lipids, proteins, and nucleic acids. During normal function of the antioxidant defense system, reduced glutathione is used by GPx to detoxify hydrogen peroxide. The tendency for GPx activity to decrease after competition may denote the effect of competition inducing oxidative stress on horses. Furthermore, as has been previously indicated, the observed increase of LPO plasma levels in sport horses after competition is an indicator of competition-induced lipid peroxidation.

As has been previously mentioned, free radicals have been implicated as mediators of exercise-induced cellular dysfunction. On the other hand, it is accepted that mitochondrial production of oxygen-derived radicals could be increased during exercise and this fact could be a major mechanism for the exercise-related increase in oxidative damage. Our finding of increased plasma LPO levels after competition support the hypothesis of augmented oxidative stress and damage with exercise, and it is in accordance with previous studies from other groups.

In conclusion, competition produced evidence of lipid peroxidation and changes in antioxidant enzymes. Competition-induced oxidative stress is linked with increased lipid peroxidation. These observations provide evidence that competition increases oxygen consumption and causes a disturbance of intracellular pro-oxidant-antioxidant homeostasis.

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