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Apoptosis and antioxidant status are influenced by age and exercise training in horses

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ABSTRACT: Eight mature (12 ± 2 yr; MAT) and 5 older (22 ± 2 yr; OLD) Standardbred mares were used to test the hypothesis that aging and exercise training would alter apoptosis in white blood cells and antioxidant status. The horses were housed indoors overnight (16 h/d) in 3 m × 3 m stalls and were turned out in a drylot during the day. They were fed a diet consisting of total mixed ration, hay cubes fed ad libitum or an equine senior diet plus grass hay. Horses were trained for 20 to 30 min/d, 3 to 5 d/wk for 8 wk at a submaximal work intensity between 60 to 70% of maximal heart rate. A graded exercise test (GXT; stepwise test until exhaustion) was performed before (GXT1) and after (GXT2) the 8 wk of training. During the GXT, blood samples and heart rate were taken at rest, 6 m/s, fatigue, and at 5 and 60 min postfatigue. Fatigue plasma lactate concentration was greater in MAT (19.3 ± 1.5 at 10 m/s) compared with the OLD (10.9 ± 1.2 mmol/L at 9 m/s; P = 0.008) horses. There was no effect of age or training on plasma lipid hydroperoxide (LPO) concentration. However, there was a positive correlation between LPO and plasma lactate concentration (r = 0.27, P = 0.006) during acute exercise. There was a greater concentration of total glutathione after GXT1 than after GXT2 (111.8 ± 5.0 vs. 98.6 ± 3.4 μM, respectively; P = 0.0002) for both age groups. Apoptosis was less (P = 0.002) in white blood cells of the MAT vs. the OLD group. These results demonstrate that older horses are under similar amounts of oxidative stress, measured by LPO, and have similar levels of glutathione in their systems compared with mature horses. The observation that more glutathione was needed during GXT1 for both groups of horses indicates that training helps horses adapt their system for the intense post-training exercise tests. The greater level of white blood cell apoptosis also indicates that older horses may be immune-compromised during exercise. However, research still needs to be performed regarding dietary supplementation in the aged horse.

Key words: aging, antioxidant, apoptosis, equine, exercise training, glutathione

INTRODUCTION

The link between exercise and oxidative stress, or a decrease in antioxidant status, has been well documented in horses (Chiaradia et al., 1998; White et al., 2001; Williams et al., 2004b). Oxidative stress occurs when the antioxidant defense system in the body is overwhelmed with reactive oxygen species (ROS). An increase in ROS from the mitochondria may occur due to increased exposure to oxidants from the environment, increased production within the body, or an imbalance in antioxidants (McBride and Kraemer, 1999). Aging is a natural biological process characterized by a general decline in physiological function counterbalanced by repair and maintenance factors that contribute to the longevity of the animal. Research has supported the role of ROS production as a contributor to aging (Harmon, 1956; Fukagawa, 1999; Squier, 2001). The free radical theory of aging states that long-term effects of the degenerative changes associated with aging may create an accumulation of ROS and subsequent cellular damage and apoptosis (Harmon, 1956). Aging, Alzheimer’s disease, and Parkinson’s disease (Fukagawa, 1999; Squier, 2001) are examples of degenerative processes affected by chronic ROS production in humans.

The efficiency of the antioxidant system is related to the age and average life span of a particular species.
(Cutler, 1991). Animal species that have a longer life span have more efficient antioxidant systems than their shorter living counterparts. There is no published study in horses that has determined whether antioxidant status decreases or if oxidative stress and apoptosis increase with age, or if these effects are altered with exercise. Therefore, the objective of the current study was to test the hypothesis that aging and exercise training would affect antioxidant status and the degree of white blood cell apoptosis as an indicator of immune function in otherwise normal, healthy, unconditioned horses.

**MATERIALS AND METHODS**

**General Experimental Design**

All methods and procedures used in this experiment were approved by the Rutgers University Institutional Animal Care and Use Review Board.

Before initiation of the experiment, horses were re-adapted to the Equine Exercise Physiology Laboratory and with the high-speed equine treadmill (Sato I, Sato Treadmill, Knivsta, Sweden). Each horse was also taught to exercise in a free-stall, motorized, circular, equine exercise machine (Equi-Ciser, Equi-Master Int'l., Sundre, Alberta, Canada). Once the horses were deemed ready for the protocol, they underwent a pre-training, graded exercise test (GXT1, see below) to measure maximal heart rate (HRmax) and the heart rate (HR) to velocity relationship. The acute experimental testing of the GXT was completed on all of the horses between 0800 and 1100. Data from GXT1 were used to determine the intensity of the exercise used during the training period (see below). Each horse also performed a posttraining GXT (GXT2) using the same protocol.

**Animals and Diet**

The experiment was conducted using 8 mature, healthy Standardbred mares, aged 12 ± 2 yr (MAT) and 5 healthy but older Standardbred mares, aged 22 ± 2 yr (OLD). During the 8-wk experimental period, the horses were housed indoors overnight (16 h/d) in 3 m × 3 m stalls and were turned out in approximately 2-acre exercise lots (fenced paddocks with minimal grass forage) during the day.

The MAT horses were fed a diet consisting of ad libitum, total mixed ration (TMR) hay cubes (about 18 kg·horse⁻¹·d⁻¹; Eckenberg Farms, Mattawa, WA; Gordon et al., 2006) overnight beginning at 1500; the OLD group of horses received 4.5 to 5 kg/d of an equine senior diet as needed, plus mixed grass hay twice a day at 0800 and 1500 (Table 1). The difference in the diets was necessary due to the management needed to maintain the OLD horses at a body condition score of 5 to 6 during the course of the exercise training and testing. The diet was balanced to meet or exceed the 1989 NRC requirements for horses receiving moderate exercise.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Hay cubes¹</th>
<th>Senior feed²</th>
<th>Grass hay³</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>90.4</td>
<td>88.0</td>
<td>92.8</td>
</tr>
<tr>
<td>Horse DE, Mcal/kg</td>
<td>2.7</td>
<td>2.7</td>
<td>1.9</td>
</tr>
<tr>
<td>CP, %</td>
<td>18.7</td>
<td>14.0</td>
<td>7.0</td>
</tr>
<tr>
<td>ADF, %</td>
<td>26.9</td>
<td>16.0⁴</td>
<td>37.8</td>
</tr>
<tr>
<td>NDF, %</td>
<td>35.9</td>
<td>NA</td>
<td>66.2</td>
</tr>
<tr>
<td>Nonfiber carbohydrates, %</td>
<td>33.0</td>
<td>22.0</td>
<td>20.4</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>2.1</td>
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</tr>
<tr>
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<td>0.23</td>
</tr>
<tr>
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<td>0.18</td>
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<tr>
<td>Sodium, %</td>
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<td>500</td>
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<tr>
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<td>220</td>
<td>20</td>
</tr>
<tr>
<td>Copper, ppm</td>
<td>11</td>
<td>55</td>
<td>7</td>
</tr>
<tr>
<td>Manganese, ppm</td>
<td>75</td>
<td>306</td>
<td>58</td>
</tr>
</tbody>
</table>

¹Eckenberg Farms, Mattawa, WA.
²Purina Mills Inc., St. Louis, MO.
³Based on Dairy One Inc. analysis, Ithaca, NY.
⁴Available at crude fiber, %.

All horses were adapted to their diets over a 6-wk period before the GXT1 and the 8-wk training protocol. Water and salt were provided for ad libitum consumption.

**Weight and Body Composition**

All mares were weighed weekly on an electronic scale before, throughout, and 2 wk after the study. Percent body fat was calculated every 2 wk using rump fat thickness measured using B-mode ultrasonography, on both the right and left gluteal region and the Westervelt equation (Westervelt et al., 1976):

\[
\% \text{ Body Fat} = 8.64 + (4.7 \times \text{mean rump fat thickness, cm})
\]

**Graded Exercise Tests**

Pre- and posttraining fitness variables were measured during the GXT that were performed at least 1 wk before (GXT1) and within 1 wk after (GXT2) the 8 wk of training. These data were used to determine alterations in fitness due to training and to set the intensity of training at 60 to 70% HRmax (approximately 5 to 6 m/s). Horses in both age groups were chosen at random, with 3 to 4 horses running a GXT over the course of a consecutive 4-d period. Before walking on the treadmill, horses had an introducer catheter (6 F Argon Medicinal, Athens, TX) inserted percutaneously into the right jugular vein, using sterile techniques and local lidocaine anesthesia. Horses were then led onto the treadmill, where a pressure-sensing catheter (Millar Instruments, Houston, TX) was inserted and advanced through the previously placed introducer catheter so as to position the pressure sensing manometers for the measurement of right ventricular pressure...
(McKeever et al., 1993). Verification of the position of the micromanometer catheter was performed before and after each GXT using representative blood pressure waveforms recorded on a physiological recording system (Biopac Systems, Goleta, CA). During GXT, horses ran on a fixed 6% grade at an initial speed of 4 m/s for 60 s. Treadmill speed was then increased to 6 m/s for 60 s, followed by 1 m/s increases every 60 s until they reached fatigue (McKeever and Malinowski, 1997). Fatigue was defined as the point at which the horse could not keep up with the treadmill despite humane encouragement. Heart rate was calculated by counting the right ventricular pressure waveforms at the last 10 to 15 s of each step of the GXT and at 2 min postexercise. Maximal HR was defined as the point at which no further increase in HR was seen despite an increase in treadmill speed.

Exercise Training

Before training, the horses were grouped based on fitness level, which also corresponded to the age groups, with the goal being to keep horses at the same relative submaximal work intensity (60 to 70% HRmax) during the 8-wk training program. The exercise program consisted of running in the exerciser for 8 wk. Horses initially trained 3 d/wk at 60% HRmax for 20 min. The frequency, relative work intensity, and duration were gradually increased over the course of the study, with the horses exercising at the end of the training period for 5 d/wk at 65 to 70% HRmax for 30 min, with a 10-min warm-up and 10-min cool-down period at the walk. Heart rate was checked at least once per week in each group using a HR monitor (Polar Electro Inc., Woodbury, NY).

Blood Sampling

Blood samples were taken and HR was measured at rest, 6 m/s, fatigue, and at 5 and 60 min postfatigue (5 post and 60 post). Samples were also collected in the last 15 s of each speed increase during the test to determine the lactate response to the test. Blood samples were placed into prechilled tubes containing lithium heparin (Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ) and kept on ice until analyzed. The blood was used to measure packed cell volume and total plasma protein concentration. The blood was used to measure packed cell volume and total plasma protein concentration. Packed cell volume was measured using the microhematocrit technique and total plasma protein concentration was measured via refractometry. Plasma lactate concentration was measured using the microhematocrit technique. Whole blood and analyzed for percent apoptosis as an indicator of immune function, using methods previously described (Nicoletti et al., 1991; Williams et al., 2004a). Red blood cells were analyzed for total glutathione (GSH-T; Biotech GSH-420, kit #21023, Oxis Health Products Inc., Portland, OR; interassay CV 7.0%, intraassay CV 5.6%) and cellular glutathione peroxidase (GPx; Biotech GPx-340, kit #21017, Oxis Health Products Inc.; interassay CV 4.2%, intraassay CV 5.0%), as previously described in detail (Williams et al., 2004b).

The lipid hydroperoxide (LPO) concentrations in plasma were determined using the PCA-FOX assay (Wolff, 1994; Gay et al., 1999; Gay and Gebicki, 2002). Each plasma sample was divided into a blank and a test sample. Catalase was then added to both to eliminate H2O2 interference, followed by a 2-min incubation at room temperature (Nourooz-Zadeh et al., 1994). Then Tris-(2-carboxyethyl)-phosphine-HCl was used to reduce lipid hydroperoxides in the blank sample to their organic alcohols (Andersen, 1989; Nourooz-Zadeh et al., 1994). Ultrapure water was added to the test sample followed by a 30-min incubation at room temperature. All samples then received the PCA-FOX assay reagent (100 μM xyleneol orange and 20 μM ferrous ammonium sulfate in 110 mM perchloric acid) and were incubated at room temperature for 45 min (Gay and Gebicki, 2002). The reaction mixture was then centrifuged at 10,000 × g for 10 min. The supernatant was aliquoted into a micotiter plate and read at a wavelength of 560 nM (Spectramax 340 plate-reader, Molecular Devices, Sunnyvale, CA; interassay CV 3.0%, intraassay CV 4.6%). Concentrations were determined by dividing the net absorbance by the molar extinction coefficient of lipid peroxides in perchloric acid (0.0521 um−1cm−1; Gay and Gebicki, 2002).

Statistics

Data are summarized as least squares means ± SE. Outliers were determined as being < 2 SD from the mean and then withdrawn from the analysis using Fisher’s normal deviant (z). An ANOVA with repeated measures using a MIXED model (SAS Inst. Inc., Cary, NC) was used to evaluate the effects of acute exercise (as sample), age, training, and their interactions. Day of exercise was also included in the model; however, no significance was found, so it was deleted from the final analysis. If no significant difference was found between the 2 GXT, then the data were averaged unless otherwise stated for a specific GXT. The association of antioxidant status and oxidative stress with age and performance variables was tested using Pearson’s product-moment correlation. Horse was included in the model to test for significance; if not significant, then it was removed from the model. Only significant correlations were reported. Significance was inferred when P < 0.05.

RESULTS

The MAT group had a 4.8% increase (P < 0.05) in their BW (from 500 ± 9 to 524 ± 14 kg) and body fat % (from 13 ± 0.6 to 14 ± 0.8%) over the 8-wk study. The OLD group had no difference in their pretraining and
Figure 1. Plasma lactate concentration for mature (MAT) and old (OLD) horses pre- and posttraining (GXT1 and GXT2, respectively). Fatigue plasma lactate concentration was affected by age at $P < 0.01$; the effect of training was not significant. (Lactate fatigue sample would be at 10 or 11 m/s, depending on the age group.) Lactate data for 60 min postexercise was not determined.

Posttraining BW (453 ± 16 and 461 ± 21 kg, respectively) and body fat % (12.5 ± 0.7 and 12.9 ± 0.6%, respectively).

Fatigue plasma lactate concentration (Figure 1) was greater in the MAT group of horses than in the OLD group (19.3 ± 1.5 vs. 10.9 ± 1.2 mmol/L, respectively; $P = 0.008$). There was, however, no difference between the fatigue plasma lactate data of GXT1 and GXT2 for each age group. The HR$_{\text{max}}$ was greater in the MAT (218 ± 3 bpm) vs. the OLD group (195 ± 3 bpm; $P = 0.006$) as was the velocity at HR$_{\text{max}}$ (OLD = 9 m/s$^3$ vs. MAT = 10 m/s; data not shown). Training had no effect on HR$_{\text{max}}$. Run time to fatigue did not differ between GXT1 and GXT2 for the MAT (5:41 ± 10 s) or OLD (4:15 ± 15 s) horses; however, there was a difference between age groups ($P < 0.05$).

There was no effect of age for GSH-T; however, there was a significant ($P = 0.0003$) effect of sample, with peak concentrations for each GXT occurring at the 5 postsample for each age group (Figure 2). There was also a greater concentration following GXT1 than following GXT2 (111.8 ± 5.0 vs. 98.6 ± 3.4 μM, respectively; $P = 0.0002$) for each group combined at the 5 post sample. There was a weak positive correlation of GSH-T with HR (r = 0.19, $P = 0.065$).

Sample also had an effect ($P = 0.016$) on cellular GPx, with peak activities at fatigue (Figure 3). However, there was no effect of age or training. There was, however, a positive correlation of GPx with HR (r = 0.24, $P = 0.017$).

There was no effect of age or training on plasma LPO concentration (Figure 4; GXT1: OLD = 4.48 ± 0.4, MAT = 5.04 ± 0.3; GXT2: OLD = 5.24 ± 0.6, MAT = 4.34 ± 0.2 μM). However, there was a positive correlation between LPO and plasma lactate concentration (r = 0.27, $P = 0.006$) during acute exercise.

The percent apoptosis was less ($P = 0.002$) in the MAT group than in the OLD group (Figure 5). No significant correlation was found among the antioxidants, oxidative stress measures, and age.

Figure 2. Total red blood cell glutathione (GSH-T) concentrations for pre- and posttraining (GXT1 and GXT2, respectively). The data from the mature and older horses have been combined due to lack of age group differences. The effects of sample ($P < 0.001$) and training ($P < 0.001$) were significant; the effect of age was not significant. *Indicates a difference between pre- and posttraining tests ($P < 0.05$).
DISCUSSION

A study on the equine population in the United States as of 2005 has shown that 7.6% of equids are over the age of 20, with 0.7% of those being over the age of 30 (USDA, 2006). This same study reported that the leading cause of death in horses was old age (28.9%) compared with euthanasia, colic, and other major diseases.

Until now there have been no published reports on the effect of exercise combined with aging on markers of oxidative stress and cellular apoptosis in the horse. The data from the current study indicate that older horses are under the same amount of oxidative stress and have similar levels of antioxidants in their systems as their younger, but mature, counterparts. However, one of the major findings of the present experiment was that more GSH-T was needed to help control the oxidation produced during GXT1 for both groups of horses, indicating that the training protocol helped horses adapt their system for the increased oxidation produced during the intense posttraining exercise. Another major finding was the greater level of white blood cell apoptosis, which could indicate that the older horses may be immune-compromised during exercise and could potentially require antioxidant supplementation.

Effects of Aging

In the current study, the lack of age-related differences to oxidative stress or lipid peroxidation could have been due to the fact that all of the horses were healthy and all were accustomed to the treadmill exercise tests. One could speculate that a greater volume and intensity of exercise would have been needed to evoke an exaggerated response. Interestingly, these observations contrast with those in humans that have shown that age does play a role in oxidative stress and antioxidant differences (Harmon, 1956; Karolkiewicz et al., 2003; Rousseau et al., 2006). Although many other characteristics of aging are similar between humans and horses, the variables measured herein were not.

Age can affect the response to acute exercise as well as the degree of the adaptive response of a variety of systems to training. For example, previous research determining the effect of aging on the cardiovascular response to exercise found a 24% lower maximal oxygen consumption ($\text{VO}_{2\text{max}}$) in older (22 ± 0.4 yr) horses compared with younger ones (McKeever and Malinowski, 1997). That same study also reported that the maximal
velocity and the run time during the GXT were less for the old group of horses compared with the young group. However, unlike the markers of aerobic capacity, that study noted that there were no differences in the lactate response to the GXT until 5 min postexercise with greater concentrations observed in the old group. In a similar study to the present one, it was found that horses older than the current study (27 ± 0.2 yr) had a lower HRmax and VO2max that were reached at lower velocities, along with a longer recovery time from the exercise test compared with a young and middle-aged group of horses (Betros et al., 2002). Those authors also reported that the old horses had increased VO2max and maximal velocity, and a decrease in recovery time after the exercise test after a 12-wk training regimen.

The antioxidants and oxidative stress in aging animal models has demonstrated that superoxide dismutase activity is correlated to the metabolic rate multiplied by the maximum life span of the species (Cutler, 1991). There is a 2- to 3-fold increase in damaged proteins in rat cells, increase in expired alkane hydrocarbons, and a shift in redox ratios [i.e., GSH: oxidized GSH (GSSG) and NADH:NAD+] to the more prooxidant values in these aging models as well. A recent study in humans revealed that erythrocyte GPx exhibited the greatest variation related to age and training, with GPx activity being greater in exercising elderly subjects compared with other groups of sedentary and young subjects (Rousseau et al., 2006). Those authors found that elderly subjects routinely engaged in aerobic activity also had a greater concentration of GSSG without changing the concentration of GSH-T. In the current study, we were unable to measure GSSG, but also did not find a difference in GSH-T with age. Another study of elderly male subjects compared physical activity level with measures of antioxidant and oxidative stress and found that the more active males had no difference in GPx activity but greater concentrations of reduced GSH, and lower concentrations of thiobarbituric acid reactive substances (TBARS, an indicator of lipid peroxidation, similar to LPO as measured in the current study; Karolikiewicz et al., 2003). The reduced GSH concentrations also positively correlated with level of physical activity and negatively correlated with TBARS. There is some controversy, however, as to whether lipid peroxidation is decreased by physical activity in humans. Another study found activity level had no effect on oxidants and antioxidant concentration in young vs. elderly subjects (Kostka et al., 1997), which is more similar to the results found in the current study.

Aging in horses has been associated with an increased risk of infections (Ralston et al., 1988; Austin et al., 1995; Atwill et al., 1996), with fewer references examining the overall immune response in old horses (Horohov et al., 1999, 2002; Malinowski et al., 2006). In previously published literature, there exists a link between apoptosis and oxidative stress (for reviews, see Chandra et al., 2000, and Kannan and Jain, 2000), which has also been previously linked to aging in the free radical theory of aging (Harmon, 1956). In the current study, the increased apoptosis (programmed cell death) in the WBC might indicate that immunity in the older horse may be compromised, although no other indicator of immune function was measured. This was not affected by exercise as found in a previous study in which endurance-type exercise was shown to increase the level of apoptosis in mature Arabians exercised for an average of 5 h (Williams et al., 2004a). This increase was eliminated with supplemental vitamin E and lipoic acid. Other studies have shown older horses have a decreased proliferative response to various mitogens (Horohov et al., 1999, 2002). Older horses were also found to have lower antibody titers to equine influenza virus than younger horses; however, an exercise test performed before sampling did not affect the titers (Horohov et al., 1999). That previous study did reveal that, although the immune response was decreased in older horses, they seem to resist exercise-induced changes to their immune function. This is similar to what we have seen in the current study with overall changes to antioxidant status and oxidative stress. One other study using 3 age ranges of Standardbred mares undergoing 12 wk of exercise training (3 to 4 times a wk at about 60% VO2max) found that there were differences with age and pre- and posttraining exercise tests in regards to cortisol, β-endorphins, and immune function (Malinowski et al., 2006). In that study the lymphocyte cell number was less in old horses compared with young horses; however, the number of granulocytes and monocytes were not different. That study provided similar immune function results to the previous study mentioned (Horohov et al., 1999) and the current study in terms of age and chronic and acute exercise changes.

**Effects of Exercise Training**

Due to the novel findings in the current study regarding antioxidant status and the effect of exercise training, the lack of published equine research leads us to make comparisons based on previously published human research for the reader's benefit. The results from the current study demonstrate that there was about 25% more GSH-T in the red blood cells of horses at the 5 post sample during GXT1 compared with GXT2. We theorize that the horses needed to mobilize more GSH-T to combat the ROS produced as a result of intense exercise. Furthermore, the results may indicate that an adaptation to the training protocol causes an adaptation of the ROS system for intense exercise. In contrast to the current study, one study using healthy, fit, male subjects has shown that 3 mo of exercise training for a marathon competition, consisting of 4 1-h bouts per week at 70 to 80% VO2max, resulted in a decrease in multiple antioxidants after exercise (α-tocopherol, β-carotene, retinol, uric acid, and sulphydryl groups; Bergholm et al., 1999). Total glutathione only accounted for a small portion of the sulphydryl groups, and this could be one explanation for why the results found in
the current study were opposite those found in the human study.

Another study using elderly subjects performing resistance exercise 3 times a wk for 6 mo measured the antioxidant status and lipid peroxidation during a GXT before and after the 6 mo of training (Vincent et al., 2002). An increase in GXT was found in response to the exercise training, which is similar to the current study in old and younger but mature groups of horses. The study by Vincent et al. (2002) also found that there were no resting lipid peroxidation differences due to training. However, there were significantly lower TBARS concentrations after GXT2 compared with after the GXT1, which is different than in the current study. We speculate that this difference could be due to differences in training protocol consisting of resistance training in humans over a longer period of time vs. the more aerobic exercise in horses for only 8 wk in the current study.

We did not observe a significant change in LPO due to training. This is not consistent with previous literature that has shown that plasma TBARS decreased with aerobic training in humans (long-distance runners); however, this effect was not seen with anaerobic training (wrestlers; Selamoglu et al., 2000). Another study found that elderly men training at 50 to 80% HRmax for a 16-wk period had a decrease in malondialdehyde and an increase in total antioxidant capacity and GPx (Fattores et al., 2004). However, yet another study using trained elderly women (2 h/wk at a fitness center) did not find any effect of training on TBARS (Meijer et al., 2001). This could mean that our training protocol was not aerobic enough to produce the same effect as seen with the aerobic training in the previous studies in humans with an intense training protocol. However, the results seen in the current study in horses are similar to those reports from a study by Meijer et al. (2001) that reported that the 2 h/wk in the fitness center might not have been intense enough to elicit a training effect on lipid peroxidation as found in the other studies with a more intense training protocol.

Effects of Acute Exercise

The correlation between LPO and lactate demonstrates a potential link between exercise intensity and lipid oxidation. However, even though there was a positive relationship, it should be noted that there was only a trend for a significant effect of sample on LPO when one examines the effect of GXT on oxidative stress measures. A GXT alone may not produce enough of an oxidative effect in this particular case to create a significant effect on LPO. This lack of a response may be due to the type of exercise utilized (4 to 6 min at 4 to 11 m/s). Evidence for intense exercise increasing lipid peroxidation, however, has been shown in several studies utilizing simulated race tests at or above HRmax that showed lipid peroxidation increased in postexercise samples compared with preexercise levels (Mills et al., 1996; Chiaradia et al., 1998; White et al., 2001). However, only exercising horses for a longer duration, without the added intensity, may not create an increase in oxidative stress. This speculation is supported by results from a previous study in which endurance horses exercised at a longer duration but more submaximal intensities (Williams et al., 2004). In that study, LPO did not differ between the supplemented (vitamin E or lipoic acid) and control groups or in response to the treadmill exercise test. The trend in the current study indicates that the exercise protocol used should have been more intense to elicit a significant response. Therefore, a longer-term more intense bout of exercise may be needed to exaggerate the trend found in the pretraining older horses.

Overall, these data indicate that the older horses in this study are not different from the younger but mature horses in terms of their antioxidant status and oxidative stress. However, observed alterations in immune function may suggest that old horses are more prone to infections at maintenance and after exercise. It’s interesting to note that the older horses had a greater percentage of white blood cell apoptosis, but similar levels of oxidative stress, which may indicate that the 2 processes are exclusive of each other. This allows us to be prepared to prevent such an occurrence by vaccination and a balanced diet. However, research still needs to be performed in regards to dietary supplementation in the old horse and whether that has an effect on oxidative stress measures during exercise.

LITERATURE CITED

References

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