Journal of Equine Veterinary Science 48 (2017) 93–102

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Oxidative Stress and Antioxidant Status in Standardbreds: Effect of Age and Training in Resting Plasma and Muscle

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A R T I C L E I N F O

Article history:
Received 26 May 2016
Received in revised form 25 July 2016
Accepted 26 July 2016
Available online 4 August 2016

Keywords:
Oxidative stress
Aging
Horse
Training
Antioxidant
Exercise

A B S T R A C T

The hypothesis of this study was that yearlings would have more oxidative stress as measured by malondialdehyde (MDA), total glutathione (GSH-T), glutathione peroxidase (GPx), and nitric oxide (NO) than mature mares before training, but after they would have similar levels. Ten Standardbred yearling fillies and 10 mature Standardbred mares were split into trained and nontrained groups. Horses were trained 5 days/wk for 7 weeks. Blood and muscle samples were collected at rest on weeks 0, 2, 5, 7, and 9. At week 0, the yearlings had higher muscle GSH-T and NO concentrations ($P = .03$) than the trained mares. At week 9, plasma NO concentrations were lower in trained mares than in the trained yearlings ($P = .007$). Trained mares increased muscle MDA and decreased plasma MDA concentrations from weeks 0 to 9 ($P < .01$), while all mares increased muscle NO and GSH-T concentrations by week 9 ($P < .05$). Trained mares and yearlings had increased erythrocyte GPx activity at weeks 7 and 9 and GSH-T concentration at week 7 ($P < .05$). Mares had higher lipid peroxidation and lower antioxidant status in the muscle than the yearlings prior to training. Trained mares improved antioxidant status and oxidative stress levels through training, resulting in levels similar to the yearlings.

1. Introduction

Oxidative stress is an important part of many physiological processes [1–3]. Furthermore, it is well documented in humans and rats that exercise can induce oxidative stress [4,5]. However, the production of reactive oxygen species (ROS) as a result of exercise has been linked to muscle damage and soreness [6–8], thus showing that excessive levels of ROS can be damaging to the body. The occurrence of oxidative stress as a result of exercise has been well documented in horses [9–11], and the potential for resulting damage from high levels of ROS has also been shown in horses [12,13].

An exercise training protocol or a high level of fitness could help blunt the effect of oxidative stress that occurs after acute, intense exercise. Prior equine research has not shown consistent evidence that exercise training can alter levels of antioxidants and oxidative stress markers as a horse becomes more conditioned [14–16]. This could be due to differences in training protocols (duration, intensity, and length) as well as differences in the horses (age, sex, and breed). In the young growing (adolescent) horse that is novel to an exercise training program, there is no research to date looking at oxidative stress.

Oxidative stress has been linked with the aging process [4,17,18] though the effect of oxidative stress on individuals as they go through puberty and into adulthood is not clear. It is known that the age of a horse undergoing exercise affects other physiological aspects including plasma cortisol, maximal oxygen uptake, and maximal heart rate [19–21], and to date, only one study has looked at oxidative stress in old horses [16]. It has been theorized that since children endure greater oxygen cost during exercise, as

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0737-0806/$ – see front matter
http://dx.doi.org/10.1016/j.jevs.2016.07.020

well as rely more on aerobic metabolism, that they may have higher levels of oxidative stress compared to adults [22]. One would expect that young horses would be similar and have higher levels of oxidative stress, along with psychological stress, at the beginning of an exercise training regime due to the novel exercise they were performing.

The objective of this study was to compare markers of oxidative stress in skeletal muscle and blood of horses of different ages and fitness levels. The hypothesis was that young horses would have higher levels of oxidative stress as measured by malondialdehyde (MDA), total glutathione (GSH-T), glutathione peroxidase (GPx), and nitric oxide (NO) than mature horses at rest during the beginning stages of a training protocol. After 7 weeks of training, mares and yearlings will have similar levels of oxidative stress.

2. Materials and Methods

2.1. Animals and Experimental Design

The Rutgers University Institutional Animal Care and Use Review Board approved all methods and procedures used in this experiment. Ten yearling Standardbred fillies (Y; 18 ± 2.4 months) and 10 mature Standardbred mares (M; 13 ± 2.1 years) were split into two groups; trained (T; seven mares and seven yearlings) and nontrained (NT; three mares and three fillies). Therefore, the four groups in this study were yearlings trained and nontrained (YT and YNT, respectively) and mares trained and nontrained (MT and MNT, respectively). All the mares were healthy, but unfit (not undergoing consistent forced exercise in a 2- to 3-month period prior to the study). All the yearlings were healthy, unfit, and novel to exercise; prior to the start of the study, they were acclimated to the equine exerciser and high-speed equine treadmill. All 20 horses were housed at the Rutgers University Equine Facilities on 3-acre exercise drylots. The mares remained outside continuously, while the yearlings were outside 08:00 AM-4:00 PM with the remainder of the night spent inside 4 m × 4 m box stalls. All horses were fed grain twice daily to meet additional nutrient requirements [23]. The mares were fed Ultraactive (1.1 kg/d), and the yearlings were fed ultrabroodmare/yearling (1.1 kg/d) (Pennfield Feed, Lancaster, PA) (Table 1), in addition to forage (Table 2). Nutrient intake for mares and yearlings is shown in Table 3.

2.2. Training Protocol

Trained horses were exercised 5 days/wk for a total of 7 weeks using an equine exerciser. Based on maximum heart rates determined by graded exercise tests (GXTs), the intensity of the workout averaged 50%–60% of their maximum heart rate for a duration of 20–50 minutes. Horses were split into three groups each day for training based on heart rates, with a workout goal of 50%–60% of their maximum heart rate. Resting blood and muscle samples were taken from all horses in week 0. In week 1, MT and YT horses began training. The training sessions began with a 10-minute warm up walk at 1.4 m/s and then increased to a 3 m/s trot for 20 minutes. The training

Table 1
Nutrient composition of grain for mature mares and yearlings.

Nutrient	Ultraroodmare Yearling	Ultraactive Textured
DE, Mcal/kg	2.94	3.17
CP, %	14.8	12.2
Fat, %	6	5.5
Fiber—max, %	10.1	5.1
Lysine, %	0.7	0.57
Ca—min, %	0.5	0.6
Ca—max, %	0.9	0.8
P—min, %	0.5	0.5
P—max, %	0.7	0.7
Mg, %	0.21	0.19
K, %	0.8	0.61
Na, %	0.36	0.38
Fe, ppm	163	128
Zn, ppm	206	185
Cu, ppm	64	40
Mn, ppm	134	115
Cl, %	0.53	0.6
S, %	0.27	0.21
I, ppm	0.77	0.5
Co, ppm	0.27	0.2
Se, ppm	1.17	0.86
Mo, ppm	0.8	0.6
Starch—max, %	25	40
Vitamin E, IU/kg	440	236
Vitamin A, IU/kg	23,502	17,097

Abbreviations: CP, crude protein; DE, digestible energy.
Nutrients are expressed on a 100% dry matter basis. Analyses were provided by Pennfield Feed, Lancaster, PA.

Table 2
Nutrient composition of hay for mature mares and yearlings.

Nutrient	Timothy Grass	Alfalfa Orchard Grass Mix
CP, %	8.7	16.4
ADF, %	40.5	38.1
NDF, %	62.1	52.6
NFC, %	23.2	22.8
Starch, %	0.2	1.2
WSC, %	12.4	8.9
ESC, %	7.2	6.2
Fat, %	2.1	2.3
Ash, %	5.88	9.3
TDN, %	59	56.5
Ca, %	0.71	0.60
P, %	0.23	0.34
Mg, %	0.14	0.26
K, %	1.42	2.76
Na %	0.30	0.02
Fe, ppm	66	133
Zn, ppm	17	18.5
Cu, ppm	6	9.5
Mn, ppm	24	29.5
Mo, ppm	1.8	1.55
Se, ppm[a]	0.03	0.30
S, %	0.13	0.19
Chloride ion, %	0.12	0.35
Lysine, %	0.34	0.71
DE, Mcal/kg	2.13	2.16
Vitamin E, IU/kg[a]	19.5	16.5
Vitamin A, IU/kg[a]	21,340	26,000

Abbreviations: ADF, acid detergent fiber; CP, crude protein; DE, digestible energy; ESC, simple sugars; NDF, neutral detergent fiber; NFC, nonfiber carbohydrates; TDN, total digestible nutrients; WSC, water-soluble carbohydrates.
Nutrients are expressed on a 100% dry matter basis. Analyses were performed by Dairy One DHIA Forage Testing Laboratory, Ithaca, NY, unless otherwise noted.
[a] Nutrient values were obtained from NRC (1989) estimations.