ANTIOXIDANT RESEARCH & ITS APPLICATION FOR USE IN EXERCISING HORSES

Carey A. Williams, Ph.D.
Associate Professor
Equine Science Center

Introduction

- Oxidative Stress is a hot topic:
  - Google = just under 8 million results
  - Add ‘horse’ = about 535,000
  - Try ‘antioxidant’ = 37.8 million

- Today I will highlight studies from my laboratory along with other pertinent studies of antioxidants in exercising horses

Oxidative Stress

- Reactive Oxygen Species (ROS):
  - Hydrogen peroxide (H₂O₂), Singlet oxygen (¹⁰O₂)
  - Free radicals (OH⁻)

- Oxidative stress:
  - ROS > capacity of the antioxidant system
  - Homeostatic environment compromised
  - In excess, can degrade proteins, DNA and PUFAs
  - Some oxidative stress is a good thing

O₂ + 4 e⁻ + 4 H⁺  \rightarrow  2 H₂O + CO₂

Oxidative stress is associated with:
- Exercise
- Aging
- Human Diseases
  - Diabetes
  - Alzheimer’s
  - Neurodegenerative Diseases
  - Cataract development

Defense mechanism against excess ROS
- Vitamins - E, C, A
- Enzymes – GPx, SOD
- Others – GSH, LA, CoQ₁₀

Antioxidant Mechanisms

- Measuring oxidative stress
  - Can’t measure ROS
  - Intermediates or end products
    - LPO, TBARS/MDA
    - F₂-isoprostanes
    - Redox ratios
Oxidative Stress & Antioxidant Supplementation in the Endurance Horse

Study 1: During competition (80 km), compare Vitamin E supplementation with Vitamin E + C
Study 2: During competition (80 km), what happens in eliminated horses
Study 3: Treadmill simulated endurance competition (55 km), compare vitamin E with Lipoic Acid

Introduction
- Plasma TBARS ↑ after exercise
- More in horses with low vitamin E (McMeniman and Hintz, 1992)
- A single bout of submaximal exercise does not affect plasma vitamin E
  - Intense conditioning may require supplementation (Siciliano et al., 1996)
- Vit. C may spare vit. E by reducing radicals and restoring its activity
  - Polo ponies at high exercise intensities had ↑ α-tocopherol and ascorbate (Hoffman et al., 2001)

Range of Vitamin E Intake
- Vitamin E intake: 1150 - 4700 IU/d

With ↑ E intake:
- ↓er CK (r = -0.23; P = 0.002)
- ↓er AST (r = -0.22; P = 0.003)
- ↑er α-Tocopherol (r = 0.21; P = 0.005) Williams et al., 2005

Materials and Methods
- Study 1: 46 horse and rider pairs
  - Paired and randomly divided into 2 groups
  - E -- 5,000 IU vitamin E/d
  - EC -- 5,000 IU vitamin E/d + 7 g vitamin C/d
  - 3 weeks prior to race
- 34 horses completed the race
  - 74 % completion rate
  - Results are finishers only
- TEMP, HR, CK, AST, LPO, GSH and GPx significant effect of distance (P < 0.0001) Williams et al., 2004

Ascorbate
- Plasma ASC and α-Toc:
  - PRE: 4.0 ± 0.5
  - 21: 4.5 ± 0.5
  - 56: 5.0 ± 0.5
  - 80: 5.5 ± 0.5
  - REC: 5.2 ± 0.5
  - P < 0.05 (Vit E) Williams et al., 2004

Creatine Kinase
- CK, IU/L:
  - PRE: 2.0 ± 0.5
  - 21: 3.0 ± 0.5
  - 56: 3.5 ± 0.5
  - 80: 4.0 ± 0.5
  - REC: 4.5 ± 0.5
  - P < 0.05 (Vit E) Williams et al., 2004
Similar Studies

- These findings contrast with a previous result from our laboratory
  - ↓ plasma ASC during a 80 km race
    (Hargreaves et al., 2002)
- Plasma ASC also ↓ during a race and through the season in sled dogs
  (Donoghue et al., 1993; Hinchcliff et al., 2000)
  - Season ↓ was prevented by vitamin C supplementation at 1 g/Mcal ME
    • Compared to ~ 0.3 g/Mcal ME in the present study

Conclusions and Implications

- Horses in endurance exercise are undergoing oxidative stress
  - Determined by LPO and muscle enzymes
- Antioxidant supplementation necessary?
  - Adding vitamin C to E helps maintain ASC status
- What about horses that do not finish the race?

Materials & Methods

- Study 2: 40 horse and rider pairs
  - No treatments
  - Nutritional survey and blood samples
- After ride groups were made
  - Finishers (n = 24) and Non-Finishers (n = 16)

Results

![Graph showing Log CK IU/L for finishers and non-finishers at different time points.

Materials & Methods

- Study 3: 12 Treadmill trained Arabians (n=4x3)
  - Vitamin E (5,000 IU/d), 3 wks
  - Lipoic Acid (10 mg/kg/d or ~ 50 mg/kg0.75), 2 wks
  - CON (basal diet)
- 1 horse tested per day
  - Text ~ 5 hrs

Lipoic Acid

- Structure:
  - 8-carbons
  - Dithiolane ring
  - 5-carbon tail
- Discovered in 1951
  - Conditionally essential nutrient
- Antioxidant properties:
  - Fat soluble like vitamin E
  - Water soluble like vitamin C
- Neurological symptoms (humans)
  - Diabetes and Alzheimer’s
- In rats was found to:
  - LA ↑ GSH and ↓ TBARS in various tissues (Khanna et al., 1999)
Antioxidant Interaction

2 Cys
Cystine

GSH

DHLA

Vit E

Vit E•

GSSG

Vit C

2 LA

DHA

adapted from Sen and Packer, 2000

Materials & Methods

Average HRmax:
Loop 1 = 49.8 %
Loop 2 = 53.6 %
Loop 3 = 55.7 %

Results

Antioxidant Supplementation in the Intensely Exercised Horse

Study 1: Vitamin E in various doses with interval exercise
Study 2: Superoxide Dismutase with repeated sprints
Study 3: Nutraceutical extracts with a graded exercise test
Materials & Methods

• **Study 1:**
  - 12 unfit Standardbred mares, hand dosed daily for 4 wks
    - 0, 5,000 or 10,000 IU/d dl-α-tocopheryl acetate
  - GXT to determine HRmax, performed pre-study
  - Interval Exercise Test after each TRT
  - 4 wk washout

Results

• CON significantly higher CK (P = 0.05) than MOD & HI

Materials and Methods

• **Study 2:**
  - 12 healthy Standardbred mares (6x6)
  - SOD supplementation for 6 wks
    - Cross over design
  - Repeated Sprints Exercise Test (RSET)
  - Measured blood and SF
    - Inflammation, antioxidants and oxidative stress measures

Results

• TOC increased with exercise; MOD and HI groups were significantly higher than CON at 24 hr (P < 0.01)

• BC was significantly higher (P < 0.05) in MOD vs. HI groups

Soccer Players

• 8-Isoprostanes (pg/mL) = significant Group x Trial interaction (p<.01)

• 21 d of SOD supplementation
• Pre-season training period
• Ran lactate threshold test

Williams and Carlucci, 2006
Football Players

- 8-isoprostanes (pg/mL) = significant Group x Trial interaction (p<.01)
- 7 wk of SOD supplementation
- Pre-season training period
- Wingate Aerobic Tests (bike)

Results

- No effect of SOD supplementation on cytokine transcript

Data presented as mean fold change in target gene expression
- Effect of exercise for IFNγ, IL-10, IL-1β
- No changes were detected for IL-6 and TNFα

Materials and Methods

- Study 3:
  - 9 Standardbred mares used in 2 experiments
  - GXTs 1 week apart cross-over treatments
  - Extracts administered 1 hr before exercise
    - EXP 1: Control, Ginger, Cranberry
    - EXP 2: Control, Black Tea, Orange Peel
      - Extracts developed at Rutgers’ Food Science Dept.

Results

- No treatment differences
  - Retinol was ↓ in orange peel supplemented horses than black tea or control
  - Determine if there would be a long term effect of supplementation (Smarsh et al., 2010)

- Subsequent studies looked at inflammatory cytokines and performance
  - Black tea and Cranberry ↓ inflammatory markers
  - Orange peel and Ginger ↓ recovery time
    - However Ginger increased CK
      - Streltsova et al., 2006; Liburt et al., 2010
Other Exercise Studies

**Study 1:** Aging and training on oxidative stress, antioxidants and apoptosis

**Study 2 & 3:** Oxidative stress during a 3-Day event competition

---

**Materials & Methods**

**Study 1:** Standardbreds
- 8 Young (age 12 ± 2 yrs)
- 5 Old (age 22 ± 2 yrs)
- Exercise program = equine exerciser for 8 wks
  - Submaximal work intensity between 65-70 % HRmax
  - Graded exercise test (GXT) performed before and after training period
  - Blood samples and HR were taken
    - Rest, 6 m/s, fatigue, 5 & 60 min post

---

**Results**

- Sample \((P = 0.0003)\) and Training \((P = 0.0002)\) effect

![Graph showing oxidative stress results](image)

Williams et al., 2008

- Age effect with OLD > YOUNG \((P = 0.002)\)

![Graph showing apoptosis results](image)

Williams et al., 2008

---

**Introduction**

**Study 2 & 3:** Two years (2006, 2007)

**CCI***/CCI**** International Competition
- 3-Day Eventing:
  - Dressage – grace
  - Cross-Country – endurance
  - Stadium Jumping – agility
- Over 50 % rider participation

**First report of inflammation and antioxidant status of competitive event horses**

**Other studies have found varying results in terms of HR, temp., plasma LAC, CK and AST responses**

  - (White et al., 1995, Murray et al., 2006)

**Environmental factors and nutritional status could play an important role**
Results

- Total GSH and GPx had an effect of division
  - CCI** ending higher than the CCI*** (P < 0.05)
    - Williams and Burk, 2012
- Plasma NO and TNFα ↓ over time (P < 0.01)
  - Both negatively correlated with body weight and dietary Vitamin E intake
    - Williams and Burk, 2010
- Horses were supplemented with an average of 4 supplements
  - On average they were over-supplemented 2-4 x the recommended levels of major minerals (Ca, P, K, Mg)
    - Burk and Williams, 2008

Take Home Message

- Overall these recent exercise studies have shown that oxidative stress was observed
  - Endurance, intense, and treadmill exercise
- The extent was dependent on various factors:
  - Ambient temperature, horses fitness, and work intensity
- Antioxidants beneficial to horses? Still debated
  - There have been positive reports in horses!

Thank you!

Future Studies:

- How young horses at the start of race training are effected by oxidative stress
  - Looking systemically and locally (skeletal muscle)
  - Before and after 8 weeks of training on an equine exerciser
  - Before and after an acute bout of intense exercise on a treadmill
  - LOOKING for yearlings to participate in the study! If interested please contact me today or at cwilliams@aesop.rutgers.edu or 848-932-5529

Acknowledgements:

- Rutgers faculty, staff, and students!
- Grad Students:
  - Emily Lamprecht, Ph.D.
  - Danielle Smarsh, Ph.D. candidate