

Oxidative Stress, Implications in Equine Orthopedic Disease, Insulin Resistance and a Potential Target for Therapy

Thomas Schell, D.V.M., DABVP (eq)

Timbercreek Veterinary Hospital, PC
3446 Fall Creek Ch. Rd.
Jonesville, NC 28642
(336) 526-6013
tschelldvm@gmail.com

Claudiu Neatu, M.D.

Innovatics Laboratories, Inc.
245 Park Ave., 24th Floor
New York, NY 10167
(862) 703-0315
cneatu@innovaticslabs.com

Keywords:

laminitis, navicular, insulin resistance, osteoarthritis, antioxidant, free radical, reactive oxygen species, antioxidant potential, FRAS-4, BAP, d-ROM.

ABSTRACT:

Reactive oxygen species (ROS) or free radicals are unstable molecules present within the general circulation and produced through normal physiological activities, diet, toxin exposure and general obesity. Free radicals primarily stimulate an immune response, aiding the body in elimination of invading organisms, and also serve a cellular signaling role. When found in excess, free radicals or ROS can trigger normal cellular damage leading to malfunction and possibly disease. Elevated levels of ROS have been shown to contribute to insulin resistance formation and lead to the trigger and release of further inflammatory cytokines. Detecting an elevated free radical status in horses can be accomplished utilizing the FRAS-4 device. With detection of this status in conjunction with a reduced antioxidant potential, therapy can be initiated to reverse the pathway. Elevated free radicals and a decreased antioxidant potential is common in equine orthopedic conditions as well as other metabolic conditions. Treatment of the underlying oxidative stress has been shown to aid in overall recovery including improvement in lameness. Investigation using a natural blend of herbal anti-inflammatories and antioxidants called Cur-OST® has shown to produce a dramatic response clinically in equine patients afflicted with elevated free radicals and a decreased antioxidant potential, resulting in improvement in lameness as well as insulin resistance status.

INTRODUCTION:

Oxidative stress results from an imbalance between oxidants and antioxidants in which the oxidant activity exceeds the neutralizing capabilities of antioxidants.⁴ The body is constantly bombarded by oxidizing agents via free radicals from normal physiological acts such as respiration, dietary influences and environmental factors. Free radicals vary in form and are essentially molecules or molecular fragments with one or more unpaired electrons in the outer orbit which significantly increasing their reactivity.^{5,7} The radical group includes species such as nitric oxide (NO·), superoxide (O₂^{·-}) and hydroxyl radical (OH·).⁷ Their unstable nature induces them to rob electrons from easily oxidized molecules such as fats, proteins and cellular DNA. While exchanging electrons, the donating tissue is altered in form which can lead to cellular malfunction or DNA alterations. This constant bombardment of free radicals is considered a causative factor with disease development. Various bodily states such as shock, sepsis, bacterial infections and cancer typically have increased free radical formation due to the impact of free radicals on invading organisms. However, a persistent elevation in free radical levels is potentially detrimental to overall health. The body's natural defense to free radical production is exogenous and endogenous antioxidant production. Superoxide dismutase (SOD), catalase (CAT) and glutathione-peroxidase (GPx) are naturally produced antioxidants within the body. Other secondary antioxidants typically consumed include ascorbic acid, vitamin E, alpha lipoic acid and many other naturally occurring substances.^{5,7} Antioxidants readily donate electrons to unstable free radicals, producing a stable molecule. In the process, however, they become unstable themselves until they are regenerated by other antioxidants. As a natural act of protection, free radical formation is expected and certain levels are considered healthy. However, we expect the body to maintain control through the presence of antioxidants. When antioxidant levels are

below the desired level, free radical presence persists and may elevate, which can contribute to clinical disease.

Oxidative stress has been explored in the pathogenesis of various equine diseases, including recurrent airway obstruction, chronic obstructive pulmonary disease, exercise induced pulmonary hemorrhage, equine grass sickness, equine motor neuron disease, osteoarthritis, degenerative joint disease, white muscle disease and post anesthetic myopathy.^{4,7,11} Although it presently remains unclear whether oxidative stress is the cause or the result of the disease entity, the presence of oxidative stress indicates a potential role in disease pathogenesis.¹¹

Herbs and natural antioxidants have been utilized in traditional Chinese medicine as well as western medicine for decades to improve the redox status of patients. Cur-OST® is a patent pending blend of natural anti-inflammatory herbs and antioxidants that has been shown in research trials performed by Nouvelle Veterinary, Inc. to successfully lower intra-synovial fluid levels of PGE-2 and MMP-9 in equine arthritic patients.¹⁰ The blend is top dressed onto the feed twice daily for up to 14 days for induction dosages and then reduced to once daily application for long term control. Cur-OST® was also shown in clinical trials to reduce the pain associated with equine arthritis, reducing lameness scores by up to 2 grades. In this trial, Cur-OST® was utilized as a sole therapy in those equine patients demonstrating oxidative stress, to determine overall impact clinically as well as in blood values of free radicals and overall antioxidant potential.

PURPOSE OF THE STUDY:

The purpose of this study was to evaluate free radical levels and antioxidant potential in horses afflicted with various inflammatory orthopedic conditions. The question was do elevated or persistent free radicals levels contribute to clinical signs associated with chronic inflammatory conditions, especially when in the presence of a lowered antioxidant potential? The second goal was to investigate the use of a proprietary blend of natural antioxidants and anti-inflammatories, Cur-OST®, in the therapy of these patients that were demonstrating oxidative stress. Cur-OST® is a patent pending blend of Turmeric (95% curcumin), green tea extract, mixed tocopherols, ascorbic acid, co-enzyme Q10, mushroom derived beta-glucans and molasses flavoring. The final goal was to assess the ease of use and clinical relevancy of the values determined by the FRAS-4 device.

METHODS:

Horses presented to Timbercreek Veterinary Hospital, PC for various inflammatory conditions were tested using the FRAS-4 machine which analyzes d-ROMs and BAP levels on plasma and whole blood utilizing a lithium heparin collection device. Insulin values were also determined in those horses that were believed to be affected by insulin resistance, which included the laminitic horses that presented due to the association with laminitis with insulin resistance.

FRAS-4 is a photometric analytical system for the global assessment of oxidative stress by measuring reactive oxygen metabolites (d-ROM) and the biological antioxidant potential (BAP) in whole blood or plasma samples. The principle adopted is the absorbance measurement of a sample solution in a cuvette through a monochromatic light beam; once the absorbance value has been taken, the instrument automatically provides conversion into the appropriate units using proprietary software. Both the centrifuge and the aluminum housing containing the photometric unit are temperature controlled by the system at 37°C, assuring accurate results for free radical analysis.⁸

The d-ROMs (determination of reactive oxygen metabolites) is a photometric assay for the measurement of concentration of hydroperoxides (ROOH) in biological samples. Such compounds are generated in cells by the oxidative attack of ROS on various organic substrates such as carbohydrates, lipids, amino acids, proteins and nucleotides. The d-ROMs test uses the principle of Fenton's reaction: by mixing a biological sample with an acidic buffer, the newly created transition metal ion (copper or iron) catalyzes hydroperoxide breakdown, generating new radical species such as hydroxyperoxyl (ROO[•]) and alkoxy (RO[•]). By adding a chromogen (N, N-diethyl-paraphenyldiamine) with ability to donate an electron and change color when oxidized by free radicals, and using the photometric reading available with the FRAS-4 dedicated analytical equipment, it becomes possible to quantify the amount of hydroperoxides available in the sample.⁸

The BAP (biological antioxidant potential) is a photometric assay for the measurement of antioxidant potential of blood plasma by measuring its ferric reducing ability. Ferric to ferrous ion reduction at low pH causes a color change which can be photometrically assessed using the integrated analytical device FRAS-4. In the BAP test, a small amount of blood plasma (10 µl) is dissolved in a colored solution, obtained by mixing a source of ferric ions with a chromogen (ammonium thiocyanate, NH₄SCN). After a 5-minute incubation time at 37°C, the solution will change color; the intensity of this chromatic change will be directly proportional to the plasma's ability to reduce the ferric ions to ferrous ions.⁸

The patients were placed on the Cur-OST® blend at the administration rate of one ounce of product twice daily on a small amount of grain. The patients were not administered any other modes of therapy, with the exception of corrective hoof trimming where applicable. The patients were then re-evaluated in 2 weeks for clinical signs and re-measurement of BAP, d-ROMs and insulin levels as dictated by the particular case.

RESULTS:

Normal values are considered as follows:

* d-ROMs normal range (130-150 UCARR)

* BAP (>2500 uMol/L).

* Normal insulin values ranges are considered 4-25 uU/ml.

Table 1.1 Results

Case no.	Diagnosis	Lameness score (pre)	Lameness score (post)	Insulin Level (pre)	Insulin Level (post)	d-Rom (pre)	d-Rom (post)	BAP (pre)	BAP (post)
1	Laminitis	4/5	2/5	221uU/ml	132uU/ml	275 UCARR	173 UCARR	3236 uM/L	2412 uM/L
2	Laminitis	5/5	1/5	112uU/ml	28.7uU/ml	215 UCARR	162 UCARR	1716 uM/L	2110 uM/L
3	Laminitis	4/5	2/5	29uU/ml	16.8uU/ml	262 UCARR	191 UCARR	2267 uM/L	2256 uM/L
4	Laminitis	4/5	0/5	49.5uU/ml	35uU/ml	275 UCARR	237 UCARR	1876 uM/L	2251 uM/L
5	Ruptured peroneus tertius	3/5	0/5	64uU/ml	27.5uU/ml	201 UCARR	157 UCARR	2059 uM/L	2122 uM/L
6	Bone cyst	3/5	1/5	N/A	N/A	159 UCARR	157 UCARR	1726 uM/L	2086 uM/L
7	Osteoarthritis	3/5	1/5	N/A	N/A	120 UCARR	136 UCARR	1383 uM/L	2639 uM/L
8	Chronic chough/ white line disease	2/5	0/5	N/A	N/A	331 UCARR	226 UCARR	2122 uM/L	2190 uM/L
9	Navicular	3/5	0/5	N/A	N/A	152 UCARR	117 UCARR	1911 uM/L	2110 uM/L
10	Navicular	3/5	0/5	N/A	N/A	143 UCARR	158 UCARR	1453 uM/L	1817 uM/L

Explanation of cases:

Case 1: 17 y.o. Lipizanner mare with chronic laminitis associated with insulin resistance. Bilateral front P3 rotation. Prior NSAID therapy as needed.

Case 2: 20 y.o. QH mare with acute laminitis associated with insulin resistance. Bilateral front P3 rotation. No prior NSAID therapy.

Case 3: 15 y.o. QH mare with acute laminitis associated with insulin resistance. Bilateral front P3 rotation. No prior NSAID therapy.

Case 4: 12 y.o. Paso Fino gelding with acute laminitis associated with insulin resistance. Minimal bilateral front P3 rotation. Prior therapy with NSAID as needed.

Case 5: 8 y.o. Arabian mare with peroneus tertius rupture. No current NSAID therapy.

Case 6: 3 y.o. QH mare with bone cyst in MTIII in right hind and osteophyte formation in the tibial-tarsal joint. No current NSAID therapy.

Case 7: 12 y.o. QH mare with bilateral osteoarthritis. No NSAID therapy.

Case 8: 20 y.o. Warmblood gelding with chronic cough and white line disease. No NSAID therapy.

Case 9: 20 y.o. Warmblood gelding with bilateral chronic navicular disease with evidence of bony lysis on radiograph. History of navicular bursal injections with minimal results and NSAID therapy as needed.

Case 10: 19 y.o. THB mare with chronic navicular disease. History of phenylbutazone daily for pain control.

DISCUSSION:

Oxidative stress is considered to be an imbalance between the free radical production and the antioxidant capabilities within the body. If free radical levels are elevated it can be an indication of oxidative stress, especially if the antioxidant capabilities are lowered also. Free radical production is relative to the inflammatory status and persistent elevation in those levels should lead the practitioner investigate the origin. Persistently lowered levels of antioxidant potential (BAP) might indicate a lowered ingestion of antioxidants, decreased production of endogenous antioxidants and an exhaustion of levels due to persistent free radical presence.

Equine orthopedic conditions are commonly associated with inflammation to one degree or another. Free radical production is noted to be elevated in the majority of the cases presented. The question remains whether the rise in free radicals is a result of the inflammatory condition or a primary instigator. Oxidative stress has been shown to enhance insulin resistance in several animal models.¹ In animal models of insulin resistance, plasma markers of oxidative stress were increased and when a superoxide dismutase mimetic was administered, insulin resistance was ameliorated.³ It has also been shown that the use of buthionine sulfoximine, an inhibitor of glutathione synthase that increases oxidative stress by depleting tissue glutathione, caused glucose intolerance. This suggests that excess ROS may cause insulin resistance.^{1,6,9} Another potential mechanism for the progression of insulin resistance is a decreased target tissue blood flow. Vasoconstriction via regional ROS overproduction may contribute to enhanced insulin resistance and lead to possible tissue damage.^{1,2}

Based on clinical observations detailed in our case studies, oxidative stress may play a major role in insulin resistance and associated laminitis as well as other orthopedic conditions such as osteoarthritis and navicular syndrome. Upon administration of the Cur-OST® herbal blend, the horses' lameness status improved as well as their free radical levels and antioxidant potential without the concurrent administration of prescription anti-inflammatory medications. Elevated insulin values were decreased in 14 days post therapy initiation.

Considering the ease of test performance and the potential impact of these results on therapy, it is the author's opinion that these levels should be measured on all patients presenting with clinical disease. Chronic syndromes with persistent problems may be explained by evaluating the d-ROMs and BAP levels. In many of these instances, there is evident elevation of free radical levels with concurrent lowered antioxidant potential. When evaluating these horses' post two week treatment course with Cur-OST®, it is noted that their free radical status decreased and their antioxidant potential improved. Furthermore, many of these patients demonstrated clinical improvement in their course of disease, especially the insulin resistant patients. These findings seem to suggest that free radical production above normal values may lead to an exacerbation of clinical signs, opening the possibility of the use of antioxidants in the course of therapy to help neutralize and return free radical levels towards normal.

CONCLUSION:

Oxidative stress appears to play a major role in many disease pathways of horses and may go largely undetected. The production and persistence of elevated free radical levels is not only a result of acute and chronic inflammation, but can also be the cause. Free radical production is necessary in acute situations, but becomes detrimental to overall health if they remain elevated chronically. It becomes evident after reviewing the data that many clinical diseases in horses appear to be controlled superficially with traditional therapies, however, after performing a d-ROMs and BAP analysis, clinical inflammation is still present. This implies that traditional anti-inflammatory therapy targets inflammatory proteins only, thus leaving an elevated free radical level which can further antagonize and create more inflammation. Given their persistent levels associated with chronic diseases such as diabetes, cancer, arthritis and other inflammatory conditions; supplementation with antioxidants, such as Cur-OST®, may be beneficial, especially if the antioxidant potential is decreased. Based on the trial results, it can be concluded that Cur-OST® safely and effectively reduced free radical levels and improved antioxidant potential. The mode of action can be viewed through primary and secondary antioxidant capabilities, however, Cur-OST® has been shown in a previous trial to reduced intra-synovial PGE-2 and MMP-9, which may be contributing to a decreased overall inflammatory status, improved clinical lameness and a decreased circulating free radicals.¹⁰

The FRAS-4 Analytical device has proven to be a valuable asset in our practice and its use is recommended as a measure of oxidative stress in disease animals, and also a screening tool for those patients that appear healthy. The FRAS-4 device is portable with a very small footprint, leading to the requirement of little space in the clinic laboratory or ambulatory vehicle. Testing procedures are easy, require little training and only approximately 10 minutes to complete. The information that the FRAS-4 provides in terms of health has been shown to be invaluable for potential disease control and also for disease prevention. The achievement of normal levels in all patients may help to control clinical signs attributed to various disease entities and may also allow us to reduce prescription medication dosages.

Notations:

Cur-OST® (Nouvelle Veterinary Inc; Jonesville, NC)

FRAS-4 (Innovatics laboratories; New York, NY)

Disclosure:

Dr. Tom Schell is affiliated with Nouvelle Veterinary, Inc. and the product Cur-OST®.

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