Introduction

My advisor once told me, “Exercise is to horses, like lactation is to the dairy cow.” As simplistic as it sounds, this statement has had great impact on my studies. It has made me change my view on equine exercise physiology as not just something we utilize for recreation, but something that is the core of the entire horse industry.

Early investigations into exercise were stimulated by the horse’s role in agriculture. Today the horse does not have as large of an agricultural role as it does one in sport. No matter what discipline the interest is in they all revolve around athletic performance and various ways to improve it. Some of the factors contributing to superior athletic performance include biomechanics, skeletal muscle properties, anaerobic capacity, heart size and function, gas exchange, hemoglobin concentration, genetics, proper nutrition, and other external influences.

The horse has been a unique exercise physiology model for years. Physiologists are fascinated by the fact that the horse is not just a larger version of the human athlete, but an animal with various features and limitations. In terms of superiority of the equine as an athlete over the human, the top speed of a human sprinter is about 36 km/h, where the Quarter Horse can sprint at 70 km/h for 400 m (Derman and Nokes, 1994). Horses also have a VO_{2max} (maximal oxygen uptake) and maximal stroke index (stroke volume/body weight) more than twice that of a human. Their hematocrit can increase 46 % above resting due to the unique function of the spleen releasing red blood cells at the start of intense exercise, while humans have taken to illegal blood doping to get this same effect. A horse’s heart rate can increase 10-fold from resting vs. about three- to four-fold in the elite human athlete.

Over the years an increasing number of studies have focused on the health and well being of our equine athletes. To jump higher, run faster, and exercise longer may still be at the top of some researcher’s priority list, however the losses of some top equine

athletes at the 1992 Olympics in Barcelona and the 2002 World Equestrian Games in Jerez has changed the focus of some to more welfare related issues.

Oxidative stress is one type of stress that athletes of any species deal with. Exercise increases oxygen uptake; as oxygen is used to help produce energy in the mitochondria, intermediates are produced called reactive oxygen species (ROS). These ROS normally are not a problem in the resting body because of the antioxidant defense system in place to combat an overproduction. However, sometimes ROS can become overwhelming to the antioxidant defense system and pose potential problems to cellular lipids, proteins and DNA; this is called *oxidative stress* (Clarkson and Thompson, 2000).

These following few pages will explain how and why oxidative stress is harmful to the body and how supplementation of various antioxidants (vitamin E, C and lipoic acid) can alleviate these problems. The present studies will also apply some of these theories and results to the horse to try to decrease the oxidative stress and increase the antioxidant status of the equine endurance athlete.

Literature Review

Oxidative Stress and Reactive Oxygen Species

Reactive oxygen species (ROS; see abbreviation glossary) are normally produced during the metabolism of oxygen (see Equation 1) and are oxygen-containing molecules that are more active than the oxygen contained in the air we breathe. Some of these ROS include: superoxide anion radicals ($O_2^{\bullet}$), hydroxyl radicals ($HO^{\bullet}$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). During oxidative metabolism, about 98 % of the oxygen consumed forms water and CO_2, however, about 1 to 2 % of the oxygen is not completely reduced and instead forms ROS (Chance et al., 1979).

Equation 1.

$$O_2 + 4\,e^- + 4\,H^+ \xrightarrow{O_2^{\bullet}} \xrightarrow{HO^{\bullet}} \xrightarrow{H_2O_2} 2\,H_2O + CO_2$$

Originally 1 to 2 % was the standard electron flow during mitochondrial respiration that gives rise to H_2O_2 (Chance et al., 1979). However, more recently values have been reported in the range of 0.4 to 0.8 % for heart mitochondria to produce H_2O_2 in physiological concentrations of succinate (Hansford et al., 1997). Now even more recently skeletal muscle mitochondria have been found to give rise to only 0.15 % of the flow of electrons to produce H_2O_2 under resting conditions (St. Pierre et al., 2002). The results from this study suggested that if complexes I and III in the electron transport chain of the mitochondria do produce ROS during oxidation of pyruvate, than the antioxidant defense systems are capable of scavenging most all that is produced and little to no H_2O_2 escapes into the cytosol. However, during fatty acid oxidation, ROS produced primarily from complex I can lead to H_2O_2 spilling out into the cytosol (St. Pierre et al., 2002). It is also important to point out that there is a consistent small leak of electrons to oxygen that escapes the antioxidant defense systems during normal metabolism that may be sufficient to cause accumulation and thus, oxidative damage.

Oxidation of lipids can also occur, this consists of a chain reaction consisting of three steps: chain initiation, propagation, and termination (Noguchi and Niki, 1999). The chain initiation involves the formation of a free radical. The free radical may arise from light, heat or through physiological reactions during aerobic reactions. The free radical attack on the lipid will result in lipid radicals. Lipid radicals react with oxygen molecules forming peroxyl radicals ($LOO^{\bullet}$), which react with more radicals and oxygen to propagate the chain reaction. The initiating radical may result from a $HO^{\bullet}$ or a nitrogen dioxide radical ($NO^{\bullet}$). Chain termination occurs when a lipid radical or a $LOO^{\bullet}$ is scavenged by antioxidants, or when two $LOO^{\bullet}$ react to produce non-radical ketones and alcohols.

Reactive oxygen species vary in reactive capacity; therefore their half-lives will vary given their reactivity. For example, one of the most reactive radicals is $HO^{\bullet}$, it has a half-life of 10^{-9} sec; where $NO^{\bullet}$ and $LOO^{\bullet}$ are much less reactive with a half-life of 1 to 10 s. Even the less reactive molecules are always changing in concentration making their direct measurement extremely difficult (Davies et al., 1982). In addition to the short half-life their concentration in most cells is very low and the sensitivity of most instruments is not able to detect these small levels. One exception to this is the ascorbate radical, which is a fairly stable semiquinone radical present in most mammalian tissues.

Role of Antioxidants

Antioxidants are inter-related and may prevent oxidant damage in several ways: scavenging of ROS; decreasing the conversion of less reactive ROS to more reactive ROS; facilitating repair of damage caused by ROS; and providing an environment favorable for activity of other antioxidants (Clarkson and Thompson, 2000).

Lipid peroxidation occurs in tissues with a high concentration of PUFA, such as cell and organelle membranes, lipoproteins, adipose tissue and brain. Vitamin E is the most efficient in preventing lipid oxidation in lipoproteins (Kagan et al., 1990). Membrane concentration of α-tocopherol is approximately one α-tocopherol molecule to 1000 lipid molecules. The phytyl tail of the tocopherol molecule allows the positioning of the

molecule within the membrane bilayer so that the active chroman ring lies close to the surface of the membrane (Esterbauer et al., 1991).

Vitamin E Metabolism and Transport. Vitamin E is the most common antioxidant and is a fat-soluble vitamin that is absorbed similarly to other lipids. When vitamin E is ingested it is hydrolyzed by pancreatic lipases and the tocopherols are absorbed in their free form (**Figure 1**). Bile from the liver emulsifies the tocopherols to form micelles. In the small intestine, the micelles containing the tocopherols are absorbed through the intestinal wall forming chylomicrons; these are secreted into the lymph (Kayden and Traber, 1993). In the lymph, lipoprotein lipases break down the chylomicrons and some tocopherols are transferred from chylomicrons remnants to lipoproteins or tissues. From the liver, tocopherols from the chylomicron remnants are secreted into very low-density lipoproteins (VLDL) to circulate in the plasma then are hydrolyzed to low-density lipoproteins (LDL). These LDL's readily exchange tocopherols with high-density lipoproteins (HDL) and may be transferred back to chylomicron remnants in the plasma and return to the liver (Kayden and Traber, 1993).

Tissue uptake of tocopherols depends on LDL receptors on the surface of tissue cells (Kayden and Traber, 1993). Another mode of action is thought to be by direct transfer of tocopherol across tissue cell membranes down a concentration gradient. Various tissues have either rapid or slow uptake. Rapid uptake is characteristic of plasma, RBC, spleen, and liver; where slow uptake is characteristic of heart, testes, muscle brain and spinal cord (Kappus and Diplock, 1992). Tocotrienols have a similar absorption pattern to tocopherols, but when in plasma chylomicrons, the tocotrienols are deposited in adipose tissue with triglycerides (Hayes et al., 1993). Regulation of vitamin E levels in the blood and tissues is largely due to tocopherol binding protein. Patients lacking this protein have low concentrations of α-tocopherol (Traber et al., 1993).

The liver is not the main storage organ for vitamin E, like other fat-soluble nutrients. Adipose tissue is the storage tissue for up to 90 % of the body concentrations of vitamin E, with liver and skeletal muscle accounting for much of the balance. Tocotrienols seem to accumulate in the skin and adipose tissue during clearance and before

chylomicrons remnants are taken by the liver (Hayes et al., 1993). Highest concentrations of tocopherols were found in subcellular fractions of tissues within membranous organelles such as microsomes and mitochondria, which are involved in highly oxidative-reductive actions (Kayden and Traber, 1993).

Measuring Oxidative Stress and Antioxidant Status

The only way to directly measure ROS is by electron spin resonance, using an electron spin trap to measure the radical spin adducts. Because of the millisecond half-life and near immeasurable amounts of ROS, direct measurement is rarely used. In place of the direct measures of ROS most studies use so-called "footprints" left by the rapidly disappearing ROS to more easily and invasively account for the increases in oxidative stress (Clarkson and Thompson, 2000). These indirect measures are used to predict the amount of ROS or oxidative stress occurring in the system. These include the measurement of end products, other compounds of oxidative damage to lipids, DNA or proteins, and measures of antioxidants themselves.

There is not any one method or "gold standard" in measuring oxidation in lipids or proteins, so it is recommended that at least two indirect measures be used. Indirect measures of ROS can be grouped into two different categories: antioxidants (e.g. vitamin E and C, glutathione, selenium, and antioxidant enzymes) and oxidative stress markers (e.g. thiobarbituric acid-reactive substances, isoprostanes, low-density lipoprotein [LDL] oxidation, and lipid hydroperoxides).

Antioxidants. Various antioxidants are measured in plasma, RBC, WBC, and other tissues to evaluate the animal's antioxidant status.

Vitamin E status can be estimated by measurement of tocopherols and tocotrienols in plasma or other tissues. The most common measurement is plasma α-tocopherol (TOC) by high performance liquid chromatography (HPLC; Kayden and Traber, 1993). The levels of TOC in the plasma will depend on the levels of consumption in the diet and other supplements. Horses not supplemented with vitamin E have about 2 μg/mL of plasma

TOC, where after 30 days of supplementation with 300 IU/kg dry matter intake the plasma TOC increased to greater than 3 μg/mL (Siciliano et al., 1996).

Vitamin C is generally measured as ascorbate (ASC) by HPLC; its antioxidant function is mainly to reduce TOC and peroxyl radicals. The normal range of ASC for healthy horses not supplemented with vitamin C is 6 to 10 μg/mL. Normal supplementation of vitamin C to horses is 5 g/d, which increases plasma ASC concentration 2 to 3 μg/mL (Snow et al., 1987).

Selenium (Se) status is important because it is a co-factor in various antioxidant enzymes including glutathione peroxidase (GPx). Selenium status is more challenging and expensive to measure than other antioxidants and can be measured in plasma, platelets, and red blood cells by atomic absorption (Basler and Holtan, 1981). One consideration in analyzing for Se is the large variability between different laboratories, so caution needs to be taken when comparing results of different studies. Further, geographical distribution of horses can influence the Se status in the horses due to the Se content in the soils (Basler and Holtan, 1981)

Glutathione has antioxidant roles including reducing ASC and TOC radicals. It is also a scavenger of hydrogen peroxide, which uses GPx as a catalyst, forming the oxidized glutathione. Glutathione can be measured in plasma, RBC, and WBC by HPLC or spectrophotometric methods (Smith et al., 1995). In the healthy horse concentrations of total glutathione range from about 110 to 120 mmol/mg protein in RBC and 60 to 70 mmol/mg protein in WBC (Williams et al., 2001). It is important to note that the measurement of total glutathione includes the concentrations of both reduced (GSH) and oxidized (GSSG) glutathione. During exercise it may be worthwhile to analyze both separately because GSH declines during intensive exercise and GSSG increases. Values for the GSH:GSSG ratio are also used in determining redox status (Urso and Clarkson, 2003).

Antioxidant enzymes including GPx, glutathione reductase (GR), superoxide dismutase (SOD), and catalase are also used as measures of antioxidant status and ROS production. Measurement of these enzymes is usually in terms of their activity to carry out

their specific reaction and it is measured by colorimetric assay in muscle, plasma, RBC, and WBC (Maral et al., 1977).

Oxidative stress markers. The oxidative stress markers or "accumulators" are end products of an oxidative reaction. Usually under "stress" conditions these products will accumulate to a measurable level above the "unstressed" condition.

The thiobarbituric acid-reactive substances (TBARS) assay for an estimation of lipid peroxidation in plasma is currently performed by colorimetric assay (Uchiyama and Mihara, 1978). The primary TBARS chromogen is the reaction product of thiobarbituric acid and malondialdehyde (MDA), which is generated during the procedure from the decomposition of lipid hydroperoxides in the sample. Additional MDA exists covalently bound to amino groups on proteins and is liberated for reaction with thiobarbituric acid during the analytical procedure (Uchiyama and Mihara, 1978). Plasma MDA can also be formed as a side reaction during prostaglandin biosynthesis, so it must be taken into account that MDA is also a marker for platelet activation and inflammation and not just for lipid peroxidation associated with oxidative stress.

Isoprostanes are prostaglandin-like compounds that are produced by free radical catalyzed peroxidation of arachidonic acid (independent of cyclooxygenase). The method of analysis for plasma or urine samples by gas chromatography and mass spectrophotometry or ELISA testing is highly sensitive and accurate, however, it is labor intensive and fairly expensive (Roberts and Morrow, 1997). Precautions should be taken to prevent the artificial generation of isoprostanes and samples should not be thawed unless analysis is within a few hours.

Conjugated dienes (CD) appear at the onset of lipid peroxidation and are linked to several steps in lipid peroxide degradation (Urso and Clarkson, 2003). They are measured by spectrophotometry or HPLC methods. These CD are polyunsaturated molecules having two double bonds separated by a single bond. They are unspecific to type of lipid peroxidation product formed and only 30 to 55 % of lipid peroxidation is detected by the CD measurement.

Measure of lipid peroxidation by lipid hydroperoxide (LPO) involves the oxidation of polyunsaturated lipids and allylic hydrogen abstraction followed by insertion of molecular oxygen resulting in peroxyl radicals and abstract hydrogens to form LPO. These can be determined directly by HPLC or colorimetric measure or indirectly by TBARS, MDA, iodide oxidation, and CD formation. The colorimetric assay takes into account hydrogen peroxide interference and non-LPO component produced signal by using a sample blank. Normal range of LPO in plasma in healthy adults is about 5 to 10 μM (Anon, 1998).

Other indirect methods of assessing oxidative stress include ferrous xylenol orange (FOX) assay; assay for protein carbonyls; LDL-oxidation assay; assay for hydroxy-alkenals; changes in gene expression in lymphocytes; markers of DNA damage; and many more. When trying to determine oxidative stress levels, these indirect measures are only predictions, and conclusions should be drawn only after several types of assays have been completed.

Oxidative Stress Effects on Physiological Systems

Within each physiological system, oxidative stress can be involved in different disease processes. The next few paragraphs will describe its impact on various systems.

Cardiovascular System. In the cardiovascular system even brief exposure to ROS results in a decrease in high-energy phosphates, and loss of contractile function and structural abnormalities (Parthasarathy et al., 1999). With the production of growth factors and cytokines, foam cells initiate the development of atherosclerotic plaques. The interior of human atherosclerotic lesions is a highly pro-oxidant environment that contains iron and copper ions able to catalyze ROS reactions. Oxidized LDLs are available in these lesions along with antibodies directed against these molecules. Oxidative stress in these tissues activates nuclear factor-κB (NF-κB), which induces transcription of several leukocyte adhesion molecules to help form plaques.

Respiratory System. Oxygen first comes in contact with tissues of the upper respiratory airways and then alveoli, so it is only logical that these tissues are susceptible to oxidative stress when ROS are produced in excess. The ROS contribute to cell death and lysis of sensitive cells of the lung tissue causing microvascular and alveolar disruptions, and inflammation of the lung tissue. The inflammatory state caused by the oxidative stress may affect the cellular redox potential, thus imposing a direct role in modulating the pattern of gene expression in lung tissues (Haddad, 2002). Hypoxia-inducible factor-1α (HIF-1α), activator protein-1 (AP-1) and NF-κB also play a role in lung damage by oxidative stress because they are regulated by the redox potential of the cells and changes in pO_2.

Oxidative stress is associated with chronic airway disease (Kirschvink et al., 2002) and exercise-induced pulmonary haemorrhage (Derksen, 1997) in horses. However, the relationship between oxidative stress and exercise variables is difficult to establish due to the influence of diseases such as heaves (Kirschvink et al., 2002).

Nervous System. Diseases such as Alzheimer's, Parkinson's, Huntington's, and amyotropic lateral sclerosis (ALS) are believed to have oxidative stress as a role triggering the defect or accelerating it after its onset. Thiol structures of proteins make them targets for ROS attack. When they are oxidized to dithiols they result in dimerization of proteins and loss of biological function (Sokol and Papas, 1999). These ROS can also alter the structure of cell and neuron membranes affecting the ion channels and the function of enzymes. DNA damage by ROS may lead to chromosomal alterations and strand breakage. Since brain tissue is highly susceptible to membrane lipid peroxidation severe neuronal damage can occur.

Skeletal Muscle and Other Systems. Oxidative stress in the skeletal muscles becomes especially important during times of strenuous exercise. The ROS produced during exercise attack the muscle cell membrane and organelles, as there is an increased metabolism of oxygen within the mitochondria of the muscle. The increased oxygen

occurs especially the highly oxidative muscle fibers due to their higher number of mitochondria (McArdle et al., 2002).

In calcifying cartilage of long bones the main population of cells includes chondrocytes. The PUFA are important in these chondrocytes, both structurally and physiologically for mineralizing tissues of bone for the local regulation of bone modeling. Normal chondrocytes produce ROS, when they become excessive cartilage pathology can occur (Seifert and Watkins, 1997). Chronic rheumatic disease and degenerative bone and joint diseases are linked to excessive ROS production. The ROS are also capable of degrading components of the joint and this has been implicated in the pathogenesis of equine joint disease (Dimock et al., 2001).

Immune cells are prone to oxidative injury because of the high PUFA content of in membranes that are easy targets for oxidation (Chew, 1996). Deficiency of a single antioxidant that is associated with the phogocytic process resulting in an oxidant-antioxidant imbalance has been correlated with abnormal immune function.

Skin is uniquely challenged by oxidants due to its role as a barrier and exposure to high oxygen tensions. Skin is composed of cells that are constantly exposed to severe environmental stresses such as sources ranging from radiation to environmental chemicals. The ROS have been implicated in the etiology of several chronic skin conditions including skin cancer and photoaging (Podda et al., 1996). They can damage proteins leading to cross-linking, aggregation and proteolysis, further leading to alteration of protein function involved with signaling pathways in the skin cells. Evidence of this was indicated by enzyme inactivation, formation of protein carbonyls, oxidation of protein-SH, loss of tryptophan fluorescence, and enhanced protease degradation. All of the above have been involved in UV-radiated skin (Podda et al., 1996).

Antioxidant Effects on Physiological Systems

Cardiovascular System. Various studies have shown that vitamin E treated mice were less susceptible to cardiotoxicity associated with Adriamycin (antibiotic used in most cancer treatments) and have reduced catecholamine-induced arrhythmias, as well as other

cardiomyopathic changes. Vitamin E (also vitamin C and carotenoids) decreased lipid peroxidation and reduced atherogenesis, which increases the risk of coronary heart disease (Singal et al., 1998). Clinical trials have shown that with vitamin E supplementation there is a decreased risk of coronary artery disease (Rimm et al., 1993; Stampfer et al., 1993); suppressed neutrophil-mediated ROS production and decreased lipid peroxidation in myocardial infarction (MI) patients (Herbaczynska-Cedro et al., 1995), and reduced rate of nonfatal myocardial infarction (Stephens et al., 1996).

The loss of myocytes through apoptosis is reported in infarct regions of the myocardium from patients with MI. Other injuries including ischemia-reperfusion, and chronic pressure overload also have increased apoptosis. This apoptosis can be inhibited by dihydrolipoic acid (DHLA), along with vitamin E, catalase, and SOD (Hofmann et al., 1997).

Respiratory System. Liver and lungs are the major sites of GSH metabolism. Most of the GSH in the lung is found in the mitochondria, which is used when lung cells undergo high levels of oxidative stress induced especially from cigarette smoke (a causative factor in chronic obstructive pulmonary disease). The increased oxidant burden derives from the fact that cigarette smoke contains about 10^{14} free radicals/puff and that many of these, such as tar semiquinone, which can generate H_2O_2 by the Fenton reaction, are relativelylong-lived (Pryor and Stone, 1993; Zang et al., 1995). Studies have shown that modulation of intracellular thiol status by either molecular of genetic regulation of GSH synthesis in the lungs not only buffers antioxidant potential, but may also inhibit oxidant-mediated inflammatory responses (Haddad, 2002). Depletion of GSH has also been identified as a factor in apoptosis (up-regulates Bax and p53; pro-apoptosis factors). Preventing apoptosis is especially critical during the first few hours of birth, which naturally up-regulates these pro-apoptosis factors due to the lungs' first exposure to atmospheric oxygen (Haddad, 2002).

Immune System. In general, antioxidants serve to protect cell membranes through preventing lipid peroxidation, affect production of immunomodulators like interleukin-2

(IL-2), tumor necrosis factor-α (TNF-α), and arachidonic acid, spare other antioxidants by overlapping activity and regenerating each other, and inhibit excessive apoptosis and signal transduction of various transcription factors including, NF-κB and AP-1 (Wu and Meydani, 1999).

Vitamin E is a major protector of lipid peroxidation; this is one of the possible mechanisms in which it enhances immune response. Dietary vitamin E has been shown to enhance T-cell differentiation in rats, enhance lymphocyte proliferation in mice, rats and pigs, increase helper T-cell activity, increased immunoglobulin production (Peplowski et al., 1980), and increase phagocytic ability in dairy cows and mice (Heinzerlinger et al., 1974; Gyang et al., 1984). Lambs supplemented with vitamin E had higher serum antibody titers after a challenge with an influenza virus (Reffett et al., 1988). Vitamin C supplementation lowered the incidence of scouring in calves (Cummins and Brunner, 1989), maintained normal primary and secondary antibody responses in guinea pigs (Kumar and Axelrod, 1969), and produced higher antibody titers against various bacterial infections chickens (McCorkel et al., 1980).

Carotenoids, specifically β-carotene, increased numbers of helper and inducer T lymphocytes in human adults given an oral supplement (Watson et al., 1991). Cows, pigs, and rats supplemented with β-carotene had increased mitogen-induced lymphocyte proliferation during their peripartum period (Bendich and Shapiro, 1986; Hoskinson et al., 1989; Heirman et al., 1990).

Glutathione depletion in cells suppresses mitogenic response of lymphocytes and prevents them from entering the S-phase in the cell cycle (Messina and Lawrence, 1989). Glutathione supplementation *in vitro* enhances antibody dependent cellular cytotoxicity in human neutrophils and mononuclear cells (Roberts et al., 1995), increases mitogenic response of splenocytes from mice (Noelle and Lawrence, 1981) and humans (Jeannin et al., 1995), potentiates the activity of lymphokine-activated killer cells (Liang et al., 1991), and increases IL-2 production (Jeannin et al., 1995).

Nervous System. Equine motor neuron disease (EMND) is a neurodegenerative disorder of the somatic lower motor neurons that results in a syndrome of diffuse neuromuscular disease in the adult horse. There is a significant association between EMND and vitamin E status; lower plasma levels of α-tocopherol are found in diseased horses than in control horses (Divers et al., 1997). This hypothesis of vitamin E deficiency has been replaced with the newer hypothesis that vitamin E is low due to its increased utilization of scavenging the ROS that are damaging the affected nerves.

Vitamin E had a positive effect on Alzheimer's, Huntington's and ALS patients, but no effect was found when vitamin E was supplemented to Parkinson's patients (Scheider et al., 1997). In Alzheimer's patients, vitamin E delayed the progression of the disease by about seven months (Sokol and Papas, 1999). When supplemented to Huntington's patients there was no effect on neurologic symptoms in the overall group, however, in patients in the earlier stages of the disease vitamin E supplementation alleviated some of the neurologic symptoms (Peyser et al., 1995).

Lipoic acid has been used in many studies in patients with diabetic neuropathy. In patients with diabetic neuropathy there is a 100 % increase in nerve vascular resistance and a 50 % decrease in nerve blood flow (Ziegler and Gries, 1997). Lipoic acid administered to these patients reversed these changes in a dose dependent manner. It also increased GSH levels in the sciatic nerve, meaning that the greater concentration of antioxidant present helped scavenge the ROS causing the nerve damage. Lipoic acid administration also improved neurological symptoms including pain in the extremities. Lipoic acid has also been studied in rats and humans for use in treating Alzheimer's patients (Arivazhagan and Panneerselvam, 2000; Hager et al., 2001).

Other Systems. Antioxidants, especially vitamin E, scavenge ROS produced in skeletal muscle during various diseases (white muscle disease, exertional rhabdomyolysis [ER], muscular dystrophy, and exercise fatigue) in humans, horses and various other species (Orndahl et al., 1994; Zust et al., 1996; Lofstedt, 1997). Horses with ER have lower disease incidence when supplemented with high levels of vitamin E (Valberg et al., 1993).

Antioxidants may enhance bone formation and reduce production of ROS, which contribute to bone resorption. Vitamin E supplementation leads to higher rates of bone formation increased thickness of growth plate cartilage, higher mineral apposition rate, and prevented Fe-induced impairment of bone formation in rats (Seifert and Watkins, 1997). Vitamin E also protects primary tissues of avian epiphyseal chondrocytes, lowers lactate dehydrogenase activity, and prevents alveolar bone loss in the elderly avians.

High levels of antioxidants are found in the skin, which is not surprising due to the fact that the epidermis is the first line of defense against UV-radiation (McVean et al., 1999). Antioxidants including SOD, GPx, GR, catalase, ascorbic acid, GSH, and α-tocopherol are found in greater concentrations in the epidermis than in the dermis in most species. Lipoic acid and other antioxidants including vitamin C, vitamin E, GSH, catalase, and SOD were found to inhibit UV-induced damage to skin cells (Podda et al., 1996).

The previous sections only illustrate of few of the mechanisms behind how oxidative stress can harm cells and possibly cause disease. The importance of antioxidants has only been touched here, but gives the reader a glimpse of their crucial role.

Reverse Role of ROS and Oxidative Stress

Oxidants are not always detrimental to cell survival; moderate concentrations of ROS serve as cell signaling molecules. A disruption in the oxidant/antioxidant balance could lead not only to oxidative stress, but also “reductive stress”. Recently researchers observed that certain diseases are associated with a hypoxic state that results in an increased NADH:NAD^+ ratio, which leads to a reductive cytosolic environment (Lipinski, 2002). Some substances that are considered antioxidants are actually present in their oxidized form, e.g. lipoic acid. Another example is human serum albumin; it is suggested to be an antioxidant due to its one –SH group, where the other 34 thiol groups are present in the oxidized form of as disulfides. Thus, albumin can be considered a sacrificial antireductive protein which when modified by hydroxyl radicals gives a signal to proteolytic degradation and elimination from circulation (Ghyezy and Boros, 2001).

Reductive stress is associated with these antioxidants (e.g. lipoic acid and albumin), which should actually be called “antireductants”. Reductive stress is also involved in homocysteine activation of the stress response in genes, which leads to an altered endoplasmic reticulum function (Ghyezy and Boros, 2001).

The ROS production, to some extent, is necessary for immune cells to kill pathogens and clear dead tissues, but overproduction of these compounds can cause damage to the cells themselves (Bendich, 1994). Other studies have concluded that neuronal survival requires the maintenance of the redox state at an optimal level, and above or below this point may cause reductive stress to be as damaging as oxidative stress (Ghyezy and Boros, 2001). Certain ROS have been shown to stimulate sperm function leading to increased fertility. Particularly, hydrogen peroxide was found to be higher in individuals with higher fertility rates, suggesting its positive effect on fertilization (Lipinski, 2002).

One critically important source of ROS is from the *oxidative burst* reaction of macrophages and neutrophils. In these cells, ROS produced are some of the most reactive, but are essential in combating pathogenic infections and aiding in the destruction of tumor cells (Bendich, 1994). These ROS releases are not specific to the tissues requiring destruction, however, and this may lead to damage of surrounding cells as well. The crucial issue is the balance between ROS generated for beneficial purposes and the potential for damaging reactions from overproduction or insufficient antioxidant protection.

Oxidative Stress and Exercise

Antioxidant levels and oxidative stress are mainly studied using submaximal exercise because prolonged exercise increases oxygen consumption over a greater period of time. However, repeated bouts of high intensity exercise have also been used and few studies have employed maximal exercise tests to induce oxidative stress.

Maximal exercise. Protein carbonyl concentrations increase after maximal exercise in rats and return back to normal levels almost immediately after exercise is terminated. Lipid peroxidation measures also increase with intense exercise, as shown by a 60 to 100 % increase in MDA levels in rat brain cortex (Liu et al., 2000).

Results from a study performed on Thoroughbreds immediately post-race showed an increase in TBARS (1.7 to 2.2 nmol/L) compared to pre-race levels, however no change was reported in plasma ASC concentrations (White et al., 2001). No recovery samples were taken in this study. Immediately after and 18 h after intense exercise an increase of about 25 % was shown for MDA levels in equine plasma, and an increase in total GSH was also found immediately after exercise, but returned to baseline after 18 h recovery (Chiaradia et al., 1998). A further study in horses demonstrated an increase in LPO 5 and 30 min after maximal exercise, but LPO returned to resting levels by 2 h post-race (Mills et al., 1996). Additionally no differences were reported in GSH or GSSG at any sample time after the exercise as compared to before exercise. The purine nucleotide degradation products hypoxanthine and uric acid were also measured and were found to increase after exercise and return to resting levels following 2 h of recovery (Mills et al., 1996). Erythrocyte GPx concentrations decreased in Thoroughbreds after an intense bout of galloping for 1000 m; however SOD and catalase were not changed (Ono et al., 1990).

Submaximal exercise. In humans performing in a 50 km ultramarathon ASC concentration increased by almost 35 % mid-race and doubled post-race (Mastaloudis et al., 2001). The ASC concentration remained elevated 1 h post-race and fell dramatically, to well below pre-race levels by 24 h post-race. Plasma TOC concentration also increased during the race trial, and returned to near pre-race levels 1 h post-race. By by 24 h post-race the concentrations had returned to pre-race levels. The majority of results for GSH and GSSG levels after exhaustive exercise show an increase in GSSG blood concentrations with no change to a slight decrease in GSH, this reduces the ratio of GSH to GSSG (Viguie et al., 1993; Smith et al., 1995).

A human study involving a 90 min cycle at 65 % VO_{2max} on three consecutive days showed ASC concentrations increased 9.7 % over baseline after the first day and 8.3 %

after the third day (Viguie et al., 1993). This study found no change in vitamin E concentrations after the first or third day of exercise. A progressive increase in plasma isoprostanes was reported in sled dogs running 58 km on three consecutive days (Hinchcliff et al., 2000). Plasma vitamin E concentration decreased progressively on consecutive days in these dogs, however, RBC SOD and GPx did not change in response to the exercise.

As the last few paragraphs have demonstrated, conclusions made from exercise-induced oxidative stress research should be made carefully and take into account the variation in studies. Exercise type, duration, intensity, terrain, ambient temperature and subject's fitness are just a few of the factors to consider.

Antioxidant Properties of Lipoic Acid

α-Lipoic acid and its reduced form, dihydrolipoic acid (DHLA), have received widespread attention as antioxidants with both preventative and therapeutic implications to benefit humans and experimental laboratory animals. They also have potential value in production and companion animals, which warrants investigation. α-Lipoic acid is an eight-carbon structure that contains a disulfide bond as a part of a dithiolane ring with a five-carbon tail (**Figure 2**). It is a cofactor in the conversion of pyruvate to acetyl CoA as part of the pyruvate dehydrogenase complex and also in α-ketoglutarate dehydrogenase (Reed et al., 1951). Both LA and DHLA also protect the integrity of cell membranes by interacting with antioxidants—GSH, and vitamins E and C (Packer et al., 1995).

Lipoic acid approaches the "ideal" antioxidant because it fulfills the proper criteria including free radical scavenging, interacting with other antioxidants, having metal-chelating activity, is located in aqueous and/or membrane domains repairing oxidative damage, and effecting gene expression (Packer et al., 1995).

Radical Scavenging. Two sulphydryl moieties allow for radical scavenging with both the reduced and oxidized form of LA (Dikalov et al., 1997). Radical scavenging

occurs when the reaction between the ROS and the scavenger is faster than the reaction between the ROS and the target molecule (i.e. DNA, proteins, or lipids). *In vitro* experiments are performed to examine the ROS scavenging ability of compounds.

Competition experiments involve the competition of the scavenger and a detector molecule for the ROS. When there is no scavenger available the detector molecule will produce a signal that is measured. Scavenging activity is measured by the suppression of signal when the scavenger is added.

Measuring the time course of concentration of a reactant is another method in determining scavenging ability. This method eliminates the need for the detector and uses the time for the reaction between the ROS, the scavenger or the oxidized scavenger (Dikalov et al., 1997). By these methods it was determined that DHLA can reduce hydroxyl, superoxide, peroxyl (indirectly through reaction with ascorbate), glutathione, ascorbyl and chromanoxyl (indirectly through reaction with ascorbate) radicals. Hypochlorous acid, nitric oxide and trichloromethylperoxyl ($CCl_3O_2^{\bullet}$) radicals were also scavenged by DHLA (Suzuki et al., 1991). Lipoic acid, on the other hand, can also reduce hydroxyl and peroxyl (aqueous phase only) radicals and singlet oxygen, as well as hypochlorous acid, nitric oxide and $CCl_3O_2^{\bullet}$ radicals.

Interacting with Antioxidants. Lipoic acid and DHLA are capable of interacting with other antioxidants. For example, after scavenging ROS, an antioxidant forms a radical of itself (i.e. GSH forms GSSG) because in order for it to regain its antioxidant capacity it needs to be scavenged. Lipoic acid and DHLA are able to regenerate antioxidants including glutathione, ascorbic acid, and alpha-tocopherol (indirectly). Lipoic acid has a more negative redox potential (E_o = -0.32 V) than glutathione and cysteine couples (E_o = -0.24 and –0.22 V, respectively) so it can reduce GSSG to GSH and CSSC (oxidized cysteine) to CSH, but not visa versa (Navari-Izzo et al., 2002).

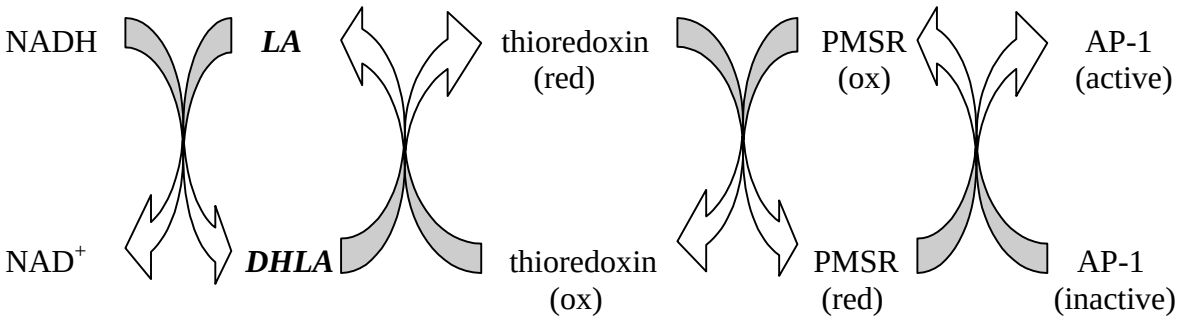
Metal Chelation. The antioxidant property of metal chelation is obtained when a complex is formed with the antioxidant and the metal, where all of the O_2 sites on the metal are occupied. This property is also obtained when the electron density is withdrawn

from the metal to the chelator, so electrons cannot be transferred to O_2. Lipoic acid and DHLA are metal chelators for various metal compounds (Biewenga et al., 1997). Lipoic acid chelates Mn^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}. Dihydrolipoic acid chelates Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Hg^{2+} and Fe^{3+}. This ability is due to the presence in the molecules of vicinal sulphur atoms and of a carboxylic group.

Solubility Characteristics. Lipoic acid is unique from other antioxidants because it is water-soluble and fat-soluble. It can have activity in the cell membrane as well as in the cytoplasm. The carboxylic acid end allows it to be more water-soluble than tocopherol, and it contains more carbon atoms than ascorbic acid so it is more lipophylic (Biewenga et al., 1997). Lipoic acid is most commonly found in nature attached to a lysine via an amide linkage. In the human diet LA is present in meat products, particularly liver and heart. The total LA was highest in the heart (3.42 nmol/g ww) and lowest in the liver (0.60 nmol/g ww), venison muscle also contains high amounts of LA (Mattulat, 1992). Most of the excreted LA was found as altered forms including beta-oxidation products, bisnorlipoate, tetranorlipoate, and beta-hydroxybisnorlipoic acid.

Repairing Oxidative Damage. An important role of an antioxidant is its ability to repair oxidative damage especially in protein with a low turnover rate. Proteins with amino acid residues such as tryptophan, histidine, tyrosine, cysteine, and methionine are susceptible to oxidation in their sulfur groups (Packer et al., 1995). This oxidation may lead to inactivation of the protein that may be an enzyme, hormone, activation or inhibition factor. One enzyme that can reduce amino acid residues (specifically methionine in this case) is peptide methionine sulfoxide reductase (PMSR). PMSR enhances the repair of oxidatively damaged proteins. The activator protein-1 (AP-1) is a protein that is influenced by the redox state of the cell. The role of LA is illustrated in Equation 2:

Equation 2.



Effects on Gene Expression. Finally, LA and DHLA have effects on gene expression including inhibition of activation of NF-κB in human T lymphocytes. By adding LA directly into the binding reaction mixture of tumor necrosis factor-α (TNF-α), NF-κB, and DNA, it inhibited the DNA binding of activated NF-κB (Suzuki et al., 1992).

Lipoic acid is reduced to DHLA in T lymphocytes; LA but not DHLA inhibits NF-κB activation by okadic acid and the DNA binding activity of activated NF-κB; however, DHLA, but not LA restores the DNA binding in a non-reducing environment (Packer et al., 1995). Apoptosis is another process where oxidation may play a role. The TNF-α induces production of reactive oxidants, which induces apoptosis. Dihydrolipoic acid prevented apoptosis in rat thymocytes stimulated with inducers of apoptosis (LA had no effect; Bustamante et al., 1995).

Practical Application. Some other properties of LA include its use as a topical antioxidant. It can penetrate the skin when applied topically and both LA and DHLA can protect against oxidative damage on the skin (i.e. UV radiation; Podda et al., 1996). Lipoic acid also has metabolic antioxidant properties. Lipoamide is an essential cofactor in mitochondrial alpha-keto acid dehydrogenase complexes. Lipoic acid is rapidly taken up by cells and reduced to DHLA, using NADH and NADPH as mediators (Packer et al., 1995).

Because of lipoic acid's role as an antioxidant in mammalian species it has been found to have implications for use in various diseases. In diabetes LA may react with

cellular sulfdryl groups involved in the regulation of insulin-stimulated glucose transport. Clinical improvement of diabetic neuropathy was also observed (Ziegler and Gries, 1997). Lipoic acid and DHLA have been shown to be effective in decreasing or preventing damage caused by ischemia-reperfusion (Serbinova et al., 1992). Various liver diseases including mushroom poisoning and alcoholic liver disease may also be influenced by LA supplementation (Packer et al., 1995). Lipoic acid and DHLA prevented HIV replication and the activation of NF-κB transcription factor, which are regulated by oxidative stress (Baur et al., 1991). Lipoic acid is also being studies as a new treatment option for Azheimer type dementia (Hager et al., 2001). Supplementing LA has found to be beneficial in a number of oxidative stress models: ishemia-reperfusion injury (Serbinova et al., 1992), diabetes (Ziegler and Gries, 1997), cataract formation (Maitra et al., 1994), radiation injury (Ramakrishnan et al., 1992), aging (Arivazhagan and Panneerselvam, 2000) and exercise (Khanna et al., 1999).

Apoptosis and Exercise

The term ‘apoptosis’ was introduced in 1972 (Kerr et al., 1972) and is Greek for “falling off or apart”. Apoptosis is also known as ‘programmed cell death’. The process of apoptosis utilizes caspases, which are cysteine aspartate-specific proteases that mediate the initiation of apoptosis through a cascade of proteins and initiation factors (Sen and Roy, 2001). The first stage in apoptosis involves cell shrinking and loss of contact with neighboring cells (**Figure 3**). Then the cell’s chromatin condenses and the cell is fragmented into membrane-enclosed structures called ‘apoptotic bodies’ (Kerr et al., 1972). Finally the macrophages engulf the body thereby removing it from the tissue without causing an inflammatory response. This process is different from necrosis, which is characterized by cell swelling, bursting, and expelling contents, which triggers an inflammatory response.

Apoptosis can be triggered by a variety of stimuli including ligation of cell surface receptors, starvation, growth factor or survival factor depletion, heat shock, hypoxia, DNA damage, viral infection, and cytotoxic/chemotherapeutical agents (**Figure 4**; Chandra et al.,

2000). Apoptosis is a key factor in the pathogenesis of many disorders. Excessive apoptosis plays a role in muscle atrophy, AIDS, and neurodegenerative diseases such as Alzheimer's and Parkinson's; whereas a depressed amount of apoptosis is a factor in some cancers, and rheumatoid arthritis (Chandra et al., 2000). Exercise induced cell apoptosis is related to the increased production of ROS that accumulate, increasing oxidative stress in the body (Phaneuf and Leeuwenburgh, 2001). Lymphocytes and skeletal muscle undergo oxidative stress during intense exercise and thus interest has arisen as to the rate of apoptosis in those cells and tissues in athletes.

Role of Free Radicals. The increased ROS production during exercise leads to an increase of apoptosis in cells. This increase can be triggered by caspase redox control, increased intracellular muscle Ca^{2+}, ATP depletion, and interacting with apoptosis inducing factors or anti-apoptotic proteins (Tan et al., 1998). Significant amounts of DNA damage may alter the expression of apoptotic proteins and initiate apoptosis. Increased oxidant production and decreased glutathione levels, can trigger mitochondria to release caspase-activating proteins, such as cytochrome c and apoptosis-inducing factor (Phaneuf and Leeuwenburgh, 2001).

Generation of ROS occurs during apoptosis, and antioxidants inhibit this generation (**Figure 5**). An antioxidant formula containing α-tocopherol, α-lipoic acid, coenzyme Q_{10}, carnitine, and selenomethionine was supplemented to healthy individuals to determine if antioxidants would decrease apoptosis in lymphocytes (Mocsa et al., 2002). After 3 wks of supplementation, individuals had an increased antioxidant status ($P < 0.05$), decreased percentage of apoptotic lymphocytes ($P < 0.01$), and decrease in ROS production ($P < 0.01$). A downfall of this study was the lack of a negative control, which makes it difficult to separate the benefit of the supplement from the factor of time. In another study, metal chelators and radical scavengers were added to rat thymocytes after induction of apoptosis (Wolfe et al., 1994). The addition of the chelators and scavengers inhibited apoptosis produced by the inducing compounds. Apoptosis also induces ROS generation in human T-cell leukemic cells (Suzuki et al., 1998). The ROS generation, along with apoptosis, is

depressed by the addition of diphenylene iodonium chloride (DPI), which inhibits ROS generating enzymes like NADPH oxidase.

The original hypothesis (Tan et al., 1998; Phaneuf and Leeuwenburgh, 2001) that ROS induced apoptosis by activating caspases is now under revision (Sen and Roy, 2001). It is believed that ROS function in apoptosis is influenced by numerous variables such as concentration, chemical type, and cellular localization. Caspases contain an active thiol group necessary for activity, which makes them susceptible to redox modification. Caspase activity may be potentiated in leukemic cells by intracellular reducing agents such as DHLA, however peripheral blood lymphocytes remained untouched in healthy humans (Sen et al., 1999). This provides a possible therapeutic use for apoptosis in leukemia patients.

Apoptosis in Lymphocytes during Exercise. Lymphocytes have a life span ranging from weeks to months (Squier et al., 1995). Apoptosis in these cells can occur at every stage of development and failures of this apoptotic process are detrimental to the body. Apoptosis has been discovered in lymphocytes first dealing with suppressed immune function during times of stress including heat, anxiety, and physical stress (Tomei et al., 1990). Lymphocyte apoptosis has important benefits including damage to the nucleic acids that may involve destruction of the viral genome in an infected cell. Also phagocytosis would prevent the spread of a virus due to the eventual rupture of the cell membrane during necrosis. Lymphocytes have several characteristics in common with tumor cells and would have to change their phenotype less than other cells to become cancerous (Squier et al., 1995). To safeguard against this lymphocyte apoptosis has a system that activates apoptosis more rapidly than in other cells.

Previous studies have shown that lymphocyte counts and function decrease in response to exhaustive exercise (Hoffman-Goetz and Pederson, 1994). In one study, thymocyte apoptosis, measured by DNA fragmentation, was induced in rats that performed two treadmill runs to exhaustion 24 h apart (Concordet and Ferry, 1993). Increased rates of apoptosis were also evident immediately after the first run and continued to be detectable 24 h post exercise.

In humans, lymphocyte apoptosis has been documented to occur immediately after and 24 h after an exhaustive exercise bout (Mars et al., 1998). In this study, endurance trained male athletes underwent a graded exercise test until exhaustion. Samples collected were analyzed by gel electrophoresis to detect DNA disruption during the apoptotic process and flow cytometry to identify percentage of apoptotic cells. The first stage is the disruption of the central core of DNA. The second stage is the peripheral aggregation or clumping of DNA, and the third stage, the condensation of DNA within the nucleus gathering toward one pole of the nucleus, resulting in single strand DNA breaking and creating a 'comet tail'. Before exercise ensued, lymphocytes were found in stages one and two. In the 24 and 48 h samples after exercise, all four stages were present in lymphocytes. Flow cytometry of lymphocytes showed an increase in all subjects from before (24.8 ± 20.2 %) to immediately (62.8 ± 17.4 %) and 24 h (86.2 ± 1.7 %) post-exercise. Using the two methods in combination allows for the determination of the stage of apoptosis experienced by the cells during exercise and recovery (Mars et al., 1998).

Apoptosis in Skeletal Muscle during Exercise. Skeletal muscle undergoes stress during exercise and the increased amount of oxygen uptake and oxidative metabolism by muscle cells increases the risk of oxidative stress and increased ROS production. Previously it was believed that damage (e.g. mechanical tears) to muscle during exercise was due to inflammatory and necrotic processes, but recent evidence has shown that it is also a result of apoptosis in muscle fibers after exercise (Carraro and Franceschi, 1997). This is the primary reason for the increases in plasma muscle enzyme concentrations after strenuous exercise. The amount of cellular damage that occurs in the muscle also depends on the nature of the contractions. Increased damage to muscle cells occurs in response to repeated eccentric or lengthening contractions rather than concentric contractions or isometric activity.

Rats that underwent spontaneous exercise (wheel running) for 16 h had higher ($P < 0.05$) levels of apoptosis in skeletal muscle immediately after (4 ± 3.5 %), and 6 h after (2.5 ± 1.8 %) exercise as compared to sedentary mice, who showed no apoptotic myonuclei (Podhorska-Okolow et al., 1999). This study also determined the expression of

apoptotic genes by Western blotting. There was a decrease in Bcl-2 expression (promotes cell survival) immediately after exercise and a decrease in Bax expression (promotes cell death) 96 h after exercise.

Thoroughbred horses exercised 5 times a wk for 3 mo were used to evaluate the occurrence of apoptosis in skeletal muscle after training. Muscle biopsies were taken from the middle gluteal 24 h after the last training session (Boffi et al., 2002). Percentage of DNA fragmentation of apoptotic cells was higher in the trained group compared to the control group (47.3 ± 17 % vs 18.6 ± 14.3 %; $P < 0.001$). The authors hypothesize that increased apoptosis during training creates a work/recovery/super-compensation cycle. This cycle is when unaccustomed muscle cells activate apoptosis to be replaced by new and stronger cells, which allows for training increases in fitness.

Apoptosis is critical in the control of cellular populations, thus regulation of apoptosis could serve many therapeutic applications in helping control certain conditions and diseases. Understanding the process of apoptosis in lymphocytes post-exercise may give us the ability to prevent depressed immune function in athletes. Understanding apoptosis in skeletal muscle could lead to different methods of training to help prevent the muscle damage in athletes.

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Glossary of Abbreviations

AP-1 = Activator protein-1
ALB = Albumin
ALS = Amyotropic lateral sclerosis
ASC = Ascorbate
AST = Aspartate aminotransferase
$CCl_3O_2^{\bullet}$ = Trichloromethylperoxyl radical
CD = Conjugated dienes
CK = Creatine kinase
CSH = Cysteine
CSSC = Oxidized cysteine
DHLA = Dihydrolipoic acid
DPI = Diphenylene iodonium chloride
EMND = Equine motor neuron disease
FOX = Ferrous xylenol orange
GPx = Glutathione peroxidase
GR = Glutathione reductase
GSH = Reduced glutathione
GSSG = Oxidized glutathione
HDL = High-density lipoproteins
HIF-1α = Hypoxia-inducible factor-1α
HPLC = High performance liquid chromatography
$HO^{\bullet}$ = Hydroxyl radical
H_2O_2 = Hydrogen peroxide
IL-2 = Interleukin-2
LA = Lipoic acid
LDL = low-density lipoprotein
LPO = Lipid hydroperoxides
$LOO^{\bullet}$ = Peroxyl radicals
MDA = Malondialdehyde
MI = Myocardial infarction
NF-κB = Nuclear factor-κB
$NO^{\bullet}$ = Nitric oxide
PI = Propidium iodide
PMSR = Peptide methionine sulfoxide reductase
PUFA = Polyunsaturated fatty acids
$O_2^{\bullet}$ = Superoxide anion radical
$^1O_2$ = Singlet oxygen
RBC = Red blood cell
ROS = Reactive oxygen species
Se = Selenium
SOD = Superoxide dismutase
TBARS = Thiobarbituric acid-reactive substances
TNF-α = Tumor necrosis factor-α
TOC = α-Tocopherol
VLDL = Very low-density lipoproteins
VO_{2max} = Maximal oxygen uptake
WBC = White blood cell

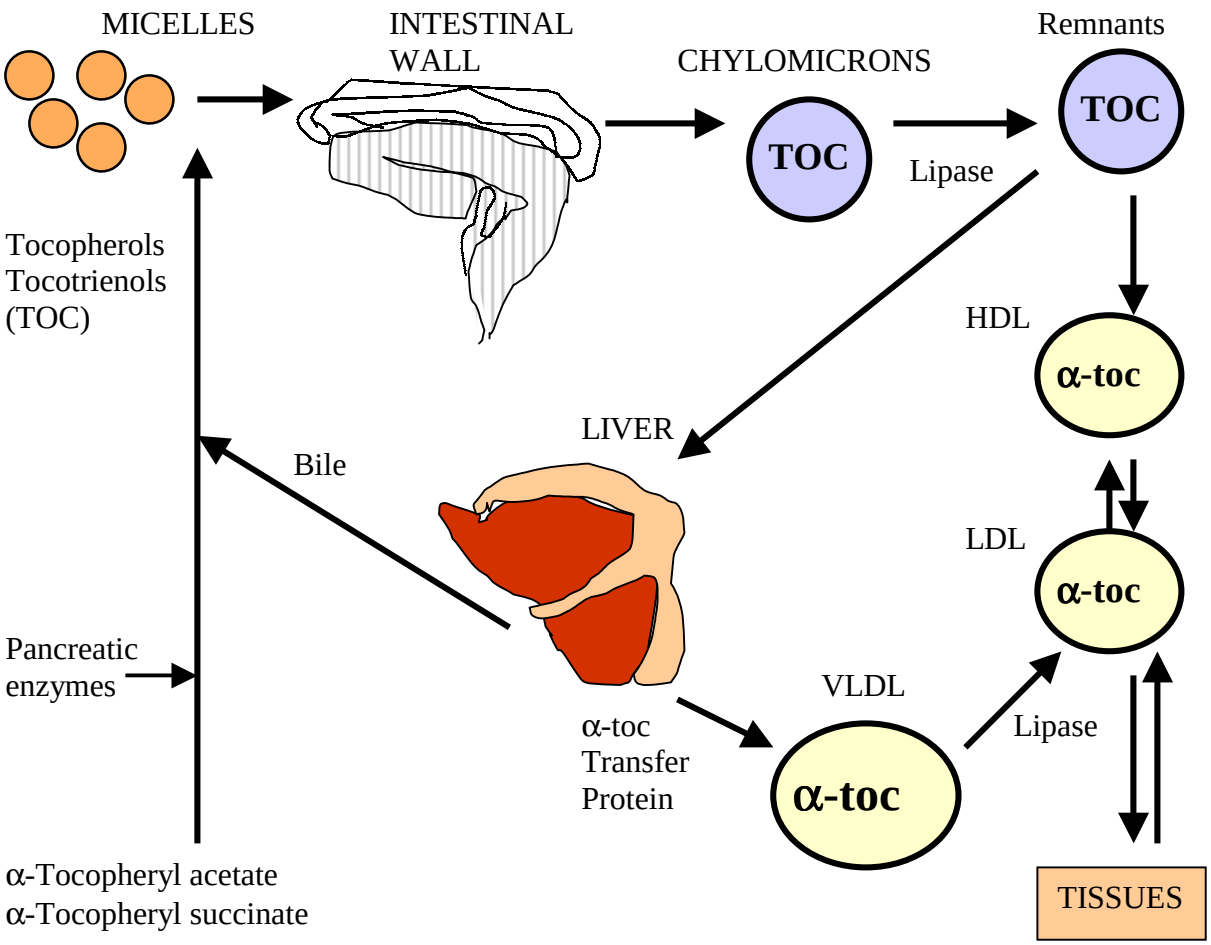
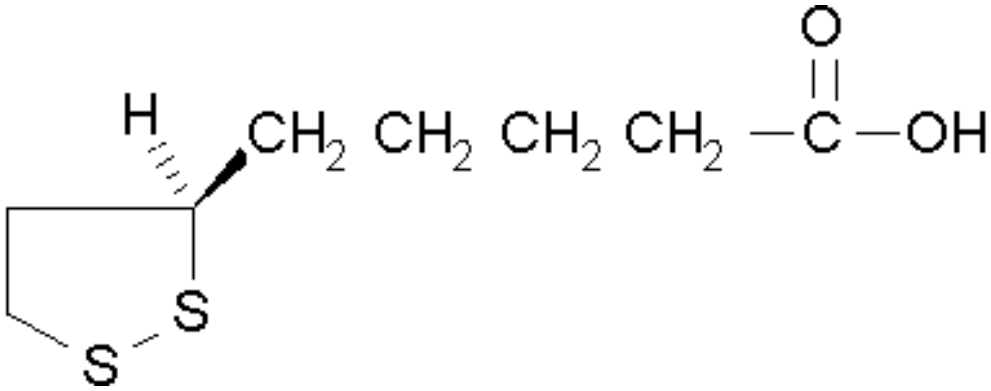


Figure 1. Absorption and transport of tocopherols as proposed by NRC (1989). Adapted from Papas, 1999.



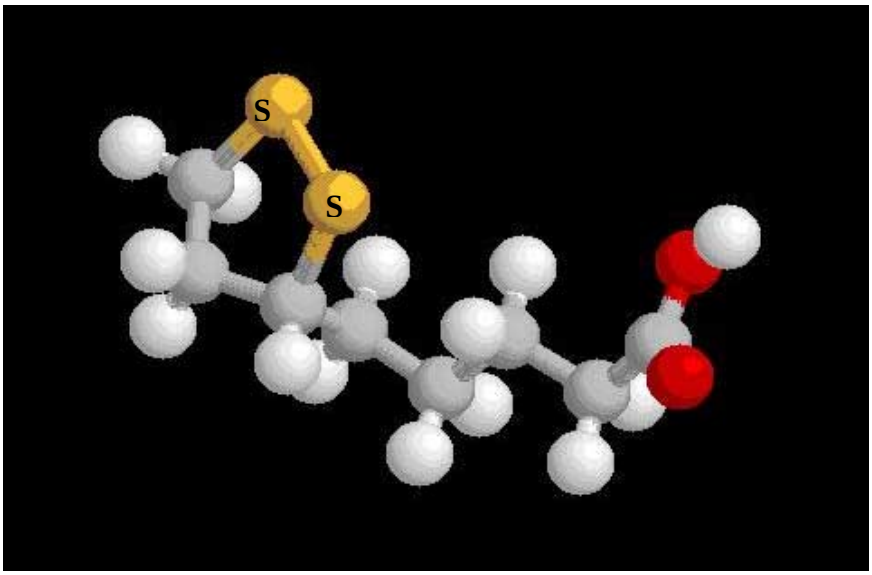


Figure 2. Lipoic acid structure. An eight-carbon structure that contains a disulfide bond as a part of a dithiolane ring with a five-carbon tail.

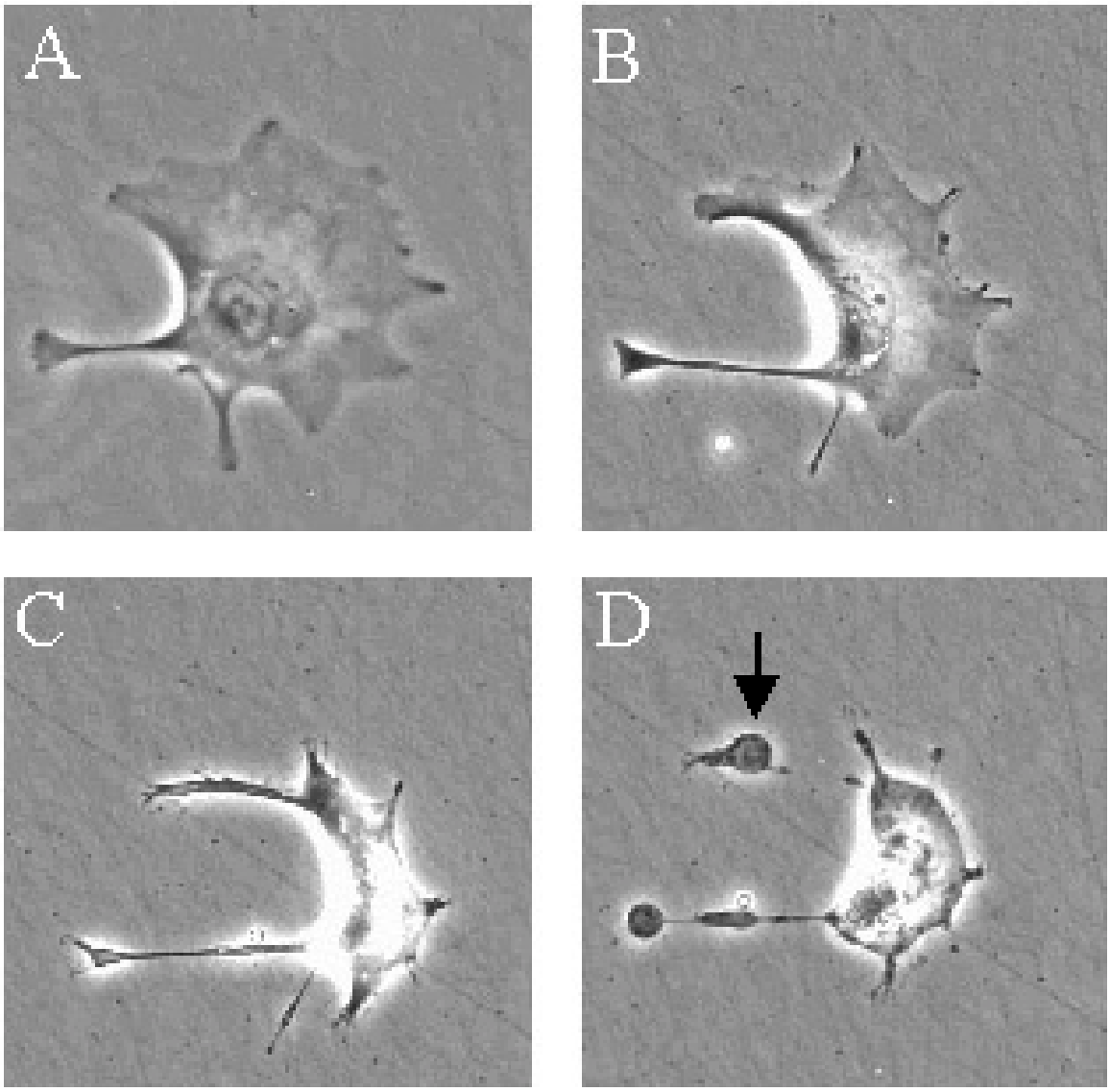


Figure 3. The apoptotic process. (A) The cytoplasm shrinks and cleavages actin filaments; (B) nuclear condensation and breakdown of chromatin and nuclear structural proteins, the nuclei look like a "horse-shoe"; (C) the cells continue to shrink and pack into a form easy for phagocytosis; and finally (D) the membrane changes start to form “apoptotic bodies” (Kerr et al., 1972).

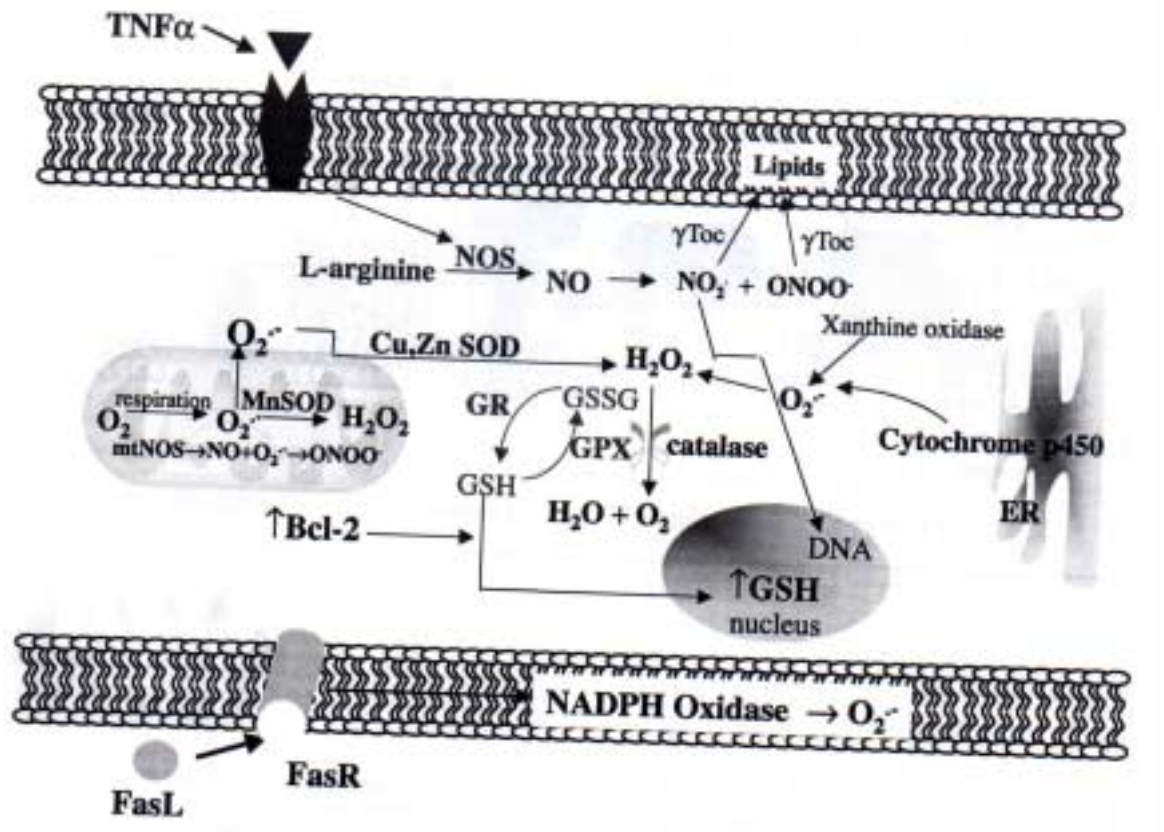


Figure 4. Apoptotic ROS sources and defense mechanisms. Sources of ROS include mitochondria, endoplasmic reticulum (ER), plasma membrane and cytosol. The conversion of H_20_2 by O_2•- relies on the glutathione (GSH) redox cycle for defense, which is present in both the cytosol and mitochondria. Enzymes in the cytosol, NADPH oxidase in the membrane, and cytochrome P450 in the ER also produce O_2•-. Bcl-2 and vitamin E (gama-tocopherols; γ-Toc) also act as antioxidants to inhibit this mechanism (Curtain et al., 2002).

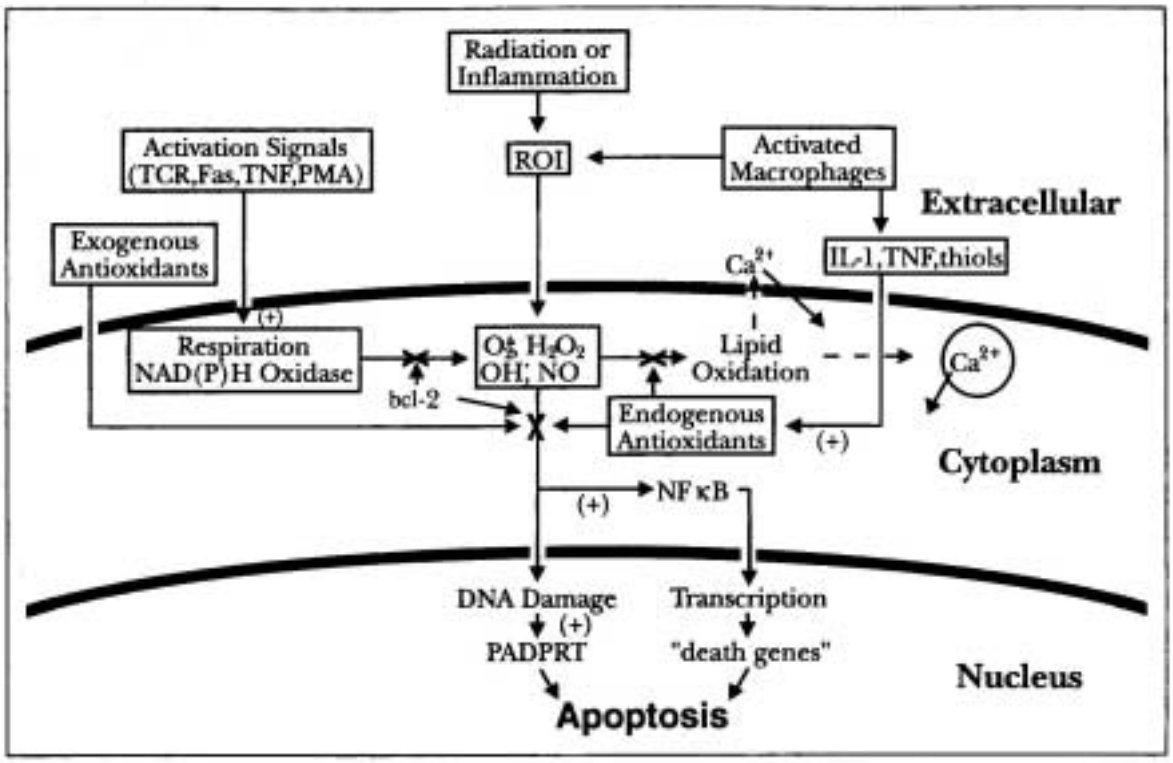


Figure 5. Interruption of the apoptotic mechanism. This pathway can by blocked by the exogenous and endogenous antioxidants as well as bcl-2 acting as an antioxidant as well (Chandra et al., 2000).

Objectives

Given the impact oxidative stress has on general health and welfare the need for investigation into various antioxidant supplements and their influence on oxidative stress and antioxidant status in the horse is warranted. The objectives for the following studies were to:

1. Evaluate the safety and antioxidant potential of lipoic acid in horses undergoing light voluntary pasture exercise.

2. Test two hypotheses: that muscle leakage may be related to oxidative stress during endurance exercise; and that these changes may be decreased by a supplement combining vitamin E and vitamin C versus vitamin E alone.

3. Compare results from the *Research Ride 2002* horses that did not finish the ride to horses that finished the ride. These results were compared to the *Old Dominion* ride and *Research Ride 2001*.

4. Calculate and compare the vitamin E content in the total diet of horses' competiting in the *Research Ride 2002* with oxidative stress measures and antioxidant status throughout the 80-km race.

5. Compare the effects of a lipoic acid or vitamin E supplement compared to horses only receiving a basal diet, on oxidative stress, antioxidant status, and apoptosis in Arabian horses exercising on a treadmill to simulate an endurance ride.