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Review Article

A REVIEW OF OXIDATIVE STRESS AND THE NRF2 PATHWAY

Promoting the body's powerful antioxidant network can affect chronic inflammation and disease at a cellular level.

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ABBREVIATIONS
Nrf2 — Nuclear factor (erythroid-derived 2)-like 2, also known as NFE2L2
OA — osteoarthritis
ROS — reactive oxygen species
SOD — superoxide dismutase

Current medical science, in both human and veterinary fields, recognizes chronic inflammation as a core issue in most diseases (1). Chronic inflammation is a key component in the progression of osteoarthritis (OA) and in many cases of musculoskeletal pain. OA is often associated with aging, but there can be several other causes or contributing factors including congenital or early developmental problems, injuries, obesity, lack of exercise, and nutrition factors.

With regard to nutrition factors, nutrigenomics is currently an emerging area of interest in the medical field. Nutrigenomics is the study of how nutrients and food components affect gene activity (both directly and indirectly) and play a regulatory role in intermediate metabolites of signaling pathways, with positive or negative effects. This means that aging and disease are affected by more than just genes. Other factors in the external environment (such as diet, exposure to chemicals, etc.) and internal factors (such as hormones and oxidative stress) can influence lifespan and *healthspan*. There is no doubt that nutrition and nutrients can have a significant influence on physiology and disease (2–4). With more research, an understanding is being developed on how optimizing nutrition can promote successful aging and resilience to inflammation and disease (5).

Helping pets to age more successfully is a prime goal in the overall health management of veterinary patients. These goals are better reached with early and proactive interventions and lifestyle choices that promote disease resistance prior to the geriatric years or the point where advanced disease states have set in. Even with patterns of disease and chronic inflammation present, we can look to new ways of assisting the body to heal and repair. One way of approaching successful aging and minimizing chronic inflammation is to support health at a cellular level. This is where the emerging science of the Nuclear factor (erythroid-derived 2)-like 2, also known as NFE2L2 or Nrf2, pathway comes in.

Nrf2 is a protein messenger (transcription factor) that exists within each cell of the body and functions as the master

regulator of endogenous antioxidant production and cellular protection (6). Activation of the Nrf2 pathway triggers the DNA to produce powerful antioxidant enzymes, anti-inflammatory proteins, and detoxification or "stress response" genes. These protective pathways are involved in virtually all areas of health from immune function to tissue repair and cognitive function as all share the common Nrf2 "switch" that enables cells to protect themselves. It appears that these pathways are affected by aging, in that the body becomes less efficient in activating the Nrf2 pathway and similar mechanisms as the body ages (7). It is now known that activation of the Nrf2 pathway can be triggered by certain foods, herbs, and exercise, as well as other lifestyle choices (e.g. intermittent fasting) (8, 9). This provides an exciting new approach to addressing health and wellness at a cellular ("root") level and also through the use of nutrigenomics.

It is important to understand inflammation and its origin in order to appreciate the potential of the Nrf2 pathway in both therapeutic and preventive medicine. At the root of inflammation and disease is *oxidative stress* (1). In fact, more than 200 diseases have been linked to oxidative stress, and research on this topic is mounting.

Diseases associated with chronic oxidative stress (**Figure 1**) (10):
Autoimmune diseases
Cancer
Cardiovascular diseases
Chronic lung disease
Diabetes
Epilepsy
Inflammatory bowel disease
Kidney disease
Neurodegenerative disease
Osteoarthritis
Rheumatoid Arthritis
Periodontal disease
Toxic liver damage

Oxidative stress is defined as the cellular damage that occurs due to free-radical affects on the cells in the body (11). Free radicals are often called reactive oxygen species (ROS). ROS are highly reactive and potentially damaging because they have an "unpaired" electron that is seeking to pair with another electron. Therefore, ROS take needed electrons from proteins, lipids, and other healthy cells, creating microscopic damage to cellular structures and leading to tissue dysfunction. The body's cells are designed to be able to protect themselves from free-radical damage via endogenous antioxidant enzymes and also

Figure 1. Oxidative stress and aging – impact on multiple organ systems. Illustration/created by: Katie Kangas & Milton Yi.

exogenous antioxidant nutrients found in foods. Antioxidants are compounds that react with and inactivate a free radical so that it cannot cause cellular damage. They do this by donating their own electrons. In this way, antioxidants help to protect every cell, tissue, and organ in the body.

The free radical theory of aging and its role in disease is not new. The hypothesis of internal radical-produced damage was first proposed by Denham Harman in 1956, with a refocus on mitochondrial-produced oxyradicals in 1972 (12). This theory holds that through a gradual accumulation of microscopic damage to our cell membranes, DNA, tissue structures, and enzyme systems, we begin to lose function and are progressively predisposed to disease (13).

The body produces these damaging free radical molecules as a normal part of living, breathing and eating/digestion. As such, this ongoing process is "normal," and the body actually does need a certain balance of free radicals for normal physiological functioning, including glucose transport, mitochondrial genesis, and muscle growth. Free radicals are also produced in the environment in the form of sunlight and becoming more abundant through pollution, toxins in food and water, poor diet and heavily processed foods. Pets and farm animals are routinely fed heavily processed foods and treated with dewormers and other insecticides to control parasites. The bottom line is, as technology and society progress, more causal factors of ROS are produced. Pets and people are being exposed to rapidly increasing amounts of environmental toxins and therefore are affected with more cellular damage. This heavy burden of ROS creates an imbalance. With too many free radicals (or too few antioxidants), the result is destruction of cell membranes and

DNA, which leads to tissue damage and a wide variety of chronic diseases, including arthritis, cancer, heart disease, gastrointestinal problems, immune dysfunction, and even obesity.

With this knowledge, medical and nutritional science turned to recommending the consumption of exogenous antioxidants in an attempt to combat oxidative stress. Over recent years both human and veterinary health markets have added multitudes of antioxidant-based products, most of which are synthetic isolates of vitamin C (ascorbic acid), vitamin E (alpha-tocopherol) and beta-carotene (a single carotenoid of many beneficial options). Unfortunately, the practice of taking antioxidants in the form of high-dose synthetic vitamin supplements is being linked to more harmful effects than benefits (14). In fact, there are multiple studies that have demonstrated increased morbidity and even mortality with high dose synthetic vitamin supplementation (15–17).

In contrast, numerous studies have clearly documented the beneficial effects of dozens of naturally occurring antioxidant nutrients when consumed as part of a healthy diet, and many nutrients and phytochemicals that possess significant antioxidant activity. Increased dietary intake of antioxidant nutrients such as vitamins C and E, minerals such as selenium, and various phytonutrients that include extracts from green tea, curcumin, grape seed, and others have all been linked to reduced rates of oxidative damage (18–23).

Indeed, the dietary intake of antioxidants is thought to help reduce the incidence of chronic diseases like heart disease and cancer. But the imbalance is difficult to affect with diet/foods alone. In other words, while there is an increase of ROS exposure, there remains a limited capacity of dietary nutrients and their activity in the body. This is because molecules of exogenous, nutrient-based antioxidants (also called direct antioxidants) can only neutralize free-radicals in a 1:1 ratio. This 1:1 relationship is referred to as "stoichiometric" scavenging (24).

Endogenous, internally produced antioxidants (also called indirect antioxidants) are far more powerful in counteracting the damaging effects of free radicals compared to dietary antioxidants (**Figure 2**) (25). These internal antioxidants, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, as well as a variety of others, are actually produced by the body's own cells and are exponentially more effective than their exogenous counterparts. This is because of the "catalytic" process of scavenging free radicals which occurs with the endogenous antioxidants, allowing them to react with and deactivate millions of free radicals every second (24).

In the mid 1990's, researchers around the world discovered Nrf2, a DNA transcription factor that "turns on" the production of SOD and other internal antioxidant enzymes. This mechanism or pathway is now called the Nrf2 pathway. The Nrf2 pathway has been referred to as the master regulator of antioxidant, detoxification, and cell defense gene expression. Nrf2 activation controls the body's expression of metabolic pathways that protect against oxidative damage triggered by injury and inflammation. One peer-reviewed article calls Nrf2 "a guardian of healthspan and gatekeeper of species longevity" (26).

As the applications of oxidative stress reduction are tremendous, numerous studies on this exciting new science are already published, and many more are underway. A recent study provided a list of diseases in which raising Nrf2 is reported to be useful in prevention and/or treatment in animal models and/or humans (27):

Cardiovascular diseases including atherosclerosis, ischemic cardiovascular disease, vascular endothelial dysfunction and heart failure
Neurodegenerative diseases including Alzheimer's, Parkinson's, Amyotrophic Lateral Sclerosis (ALS), Huntington's disease
Cancer (prevention)
Chronic kidney diseases
Metabolic diseases: Type 2 diabetes, metabolic syndrome, obesity
Several types of toxic liver disease
Sepsis
Autoimmune disease
Inflammatory bowel disease
HIV/AIDS
Multiple sclerosis
Epilepsy

More conditions are being studied beyond the extent of this list. Specifically pertaining to OA, a recent study published in Arthritis Research and Therapy found that Nrf2 has a major chondroprotective role in the progression of OA (in a mouse model) (28). Another study reported increased expression of protective factors (heme oxygenase-1) in human synovial fibroblasts with activation of the Nrf2 pathway (29). Yet another study published in Free Radical Biology Medicine reported that metabolic induction of Nrf2 redox-signaling promises to be a viable therapy for attenuating oxidative stress-mediated damage in skeletal muscle associated with physical inactivity (30).

There are numerous reports regarding the effects of oxidative stress and the benefits of Nrf2 activation as it pertains to cardiovascular diseases and chronic pulmonary diseases (31–35). Cancer

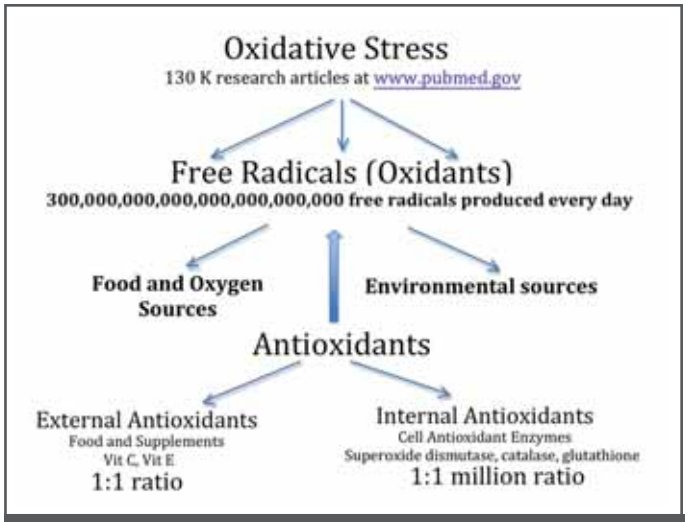

Figure 2. External vs Internal Antioxidants
Recreated from image provided courtesy of Joanne Shearer Parkin, MS, RDN. Graphic design by Milton Yi.

prevention is another field being studied for the benefits that Nrf2 may provide (31, 32, 36). Both veterinary dentistry and human dentistry fields are recognizing oxidative stress in the progression of periodontal disease and the role of Nrf2 in prevention (37, 38).

Through mounting research, it has been discovered that Nrf2 activation plays a largely protective, beneficial role in numerous diseases. This has led researchers to examine ways in which Nrf2 activation might be harnessed for health benefits, through means of exercise, diet, dietary supplements, and pharmaceuticals. To date, several medications that stimulate the Nrf2 pathway are being used or studied for the treatment of diseases that are caused by oxidative stress. These include Bardoxalone-methyl, BG-12 (dimethyl fumarate) (a), VEDA-1209, and a synthetic sulforaphane-cyclodextrin complex (b) (32). However, it is now recognized that a variety of natural products act directly upon the Nrf2 pathway, activating this life-sustaining part of DNA. These include sulforaphane (found in broccoli), turmeric, green tea extract, and many others (39–41).

A proprietary blend of 5 herbal ingredients has been developed as a dietary supplement product designed initially for people (c) followed by the development of a specific canine formulation (d). There are 5 active herbal ingredients in this particular product: milk thistle (*Silybum marianum*), turmeric (*Curcuma longa*), green tea extract (*Camellia sinensis*), ashwagandha (*Withania somnifera*), and bacopa (*Bacopa monnieri*). The synergistic effect of these 5 herbal extracts as proportioned within this patented product has been shown to effectively reduce oxidative stress in humans by an average of 40% in 30 days (42). A study in dogs using the canine product also demonstrated a reduction in oxidative stress as evidenced by increased production of catalase enzymes as well as clinical improvement in mobility and cognitive function (43). This author has been using this nutritional/dietary supplement clinically in dogs and cats for more than 3 years and has seen numerous positive results. It is also frequently used in horses. Typical responses may include increased energy, improved mobility, improved cognitive function or other specific parameters. Multiple independent and peer-reviewed studies have shown this particular supplement to be beneficial in many different applications. There are peer-reviewed studies regarding its applications in cardiovascular disease, skin cancer, cancer chemoprevention, periodontal disease, osteoarthritis and more (44 - 49).

In summary, as numerous diseases and degenerative conditions are linked to oxidative stress, affecting activation of the Nrf2 pathway allows a fundamental approach to affect and improve health at the cellular level. This is beneficial from both a therapeutic and a preventive standpoint. A recent review article stated, "We may be on the verge of new literature on health effects of Nrf2 which may well become the most extraordinary therapeutic and the most extraordinary preventative breakthrough in the history of medicine" (50). The researchers went on to say, "It is our opinion that raising Nrf2 is likely to be the most important health promoting approach into the foreseeable future (50)."

Endnote

a. Tecfidera®, Biogen, Research Triangle Park, NC.
b. Suforadex®, Evgen Pharma, Liverpool L3 5RF, UK
c. Protandim®. LifeVantage, Sandy, UT.
d. Canine Health®. LifeVantage, Sandy, UT.

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