IN TRO D U CTIO N
As a physiologic experience, life is stress. Or perhaps more pertinently, life is oxidative stress. Although oxidative stress has been implicated in a growing number of pathological conditions — from cancer and atherosclerosis, to chronic fatigue syndrome and neurodegenerative diseases — it defines not so much a disease entity as a state in which the body's antioxidant defenses are insufficient to neutralize compounds called reactive oxygen species (ROS). These free radicals are highly unstable molecular species produced during basic metabolic functions ranging from mitochondrial energy generation (oxidative phosphorylation) and immune function (neutrophilic phagocytosis) to hepatic detoxification of pollutants present in air, water and food.

The instability of free radicals comes from the unpaired electrons that are an inherent part of their molecular structure. These electrons are highly reactive, and are capable of interacting with and altering any compound in their immediate environment. To counteract the generation of free radicals and the damage that results from their activity, cells utilize antioxidants - compounds that donate their electrons to stabilize free radicals. Examples include dietary antioxidants like the proanthocyanidins found in blueberries and bioflavonoids found in citrus fruits, as well as the antioxidant enzymes superoxide dismutase, glutathione peroxidase and catalase.

Beyond their ability to scavenge free radicals, antioxidants have also been shown to blunt the production of compounds which create inflammation in the body. Scientific research is increasingly demonstrating the impact that chronic inflammation has in the development of age-related diseases like diabetes, cardiovascular and autoimmune diseases, and cancer. Because the inflammatory process creates oxidative stress and reduces cellular antioxidant capacity, much investigative focus is also being given to the role that antioxidant support plays in disease prevention and healthy aging. Because antioxidant need can vary significantly between individuals, laboratory assessments have been developed to assist clinicians in evaluating the dynamic balance between antioxidant reserve and oxidative stress in individual patients (their reduction/oxidation or "redox" balance). These tools allow the practitioner to pinpoint imbalances that increase the risk for or worsen chronic illness. In this way, they serve as a springboard for customized intervention in areas of nutrition, dietary supplement support and lifestyle/stress reduction.
**ANTIOXIDANT RESERVE**

**Glutathione**
Glutathione (GSH) is the most potent endogenous antioxidant produced by the body. This critical compound is composed of the amino acids cysteine, glutamic acid and glycine, and plays a central role in resistance to oxidative stress. Among its many roles, GSH functions as an intracellular antioxidant as well as a detoxifying agent for numerous xenobiotics, such as the organochlorides found in plastics and pesticides or the potent endocrine disruptors, polychlorinated biphenyls.

GSH deficiency has been implicated in the pathogenesis of many disorders, including heart disease, stroke, diabetes, Alzheimer’s and Parkinson’s disease, AIDS, cancer, and inflammatory bowel disease. Recent findings from Stanford University also reveal significantly decreased white blood cell GSH in patients with mitochondrial disease, indicating depleting antioxidant defenses and metabolic stress. Health and longevity are both positively associated with blood GSH levels. GSH synthesis is upregulated during oxidative stress and inflammation, an important defense mechanism but also one that can deplete reserves if not replenished. Decreases in whole blood GSH reflect similar decreases in tissues, thus serving as an indicator of overall GSH status. In fact, there is a linear correlation between GSH and natural killer cell function.

**Total Antioxidant Capacity**
Total Antioxidant Capacity (TAC) assesses the ability of an individual’s blood specimen to inhibit an oxidation reaction induced in a laboratory setting. In other words, the TAC test reveals the power of all antioxidants in a patient’s blood to neutralize free radicals. An overall decrease in TAC suggests oxidative stress, hence a need for greater antioxidant protection. Lower TAC has noted in conditions such as cancer, metabolic syndrome, heart disease, hypertension, sepsis, inflammatory bowel disease, major depression, fibromyalgia, and sulfite excess.

Most antioxidant molecules become pro-oxidants in the process of being used as reducing agents, and are subsequently restored to their reduced state by other antioxidants (e.g., GSH helps restore antioxidant status to vitamins C and E). Thus, the neutralization of ROS depends on a dynamic interaction between individual components, including vitamins A, E and C, beta-carotene, GSH, and several antioxidant enzymes. Because antioxidants work synergistically, measurement of a total antioxidant response provides a more reliable analysis of antioxidant capacity than the measurement of single antioxidants.

**Cysteine**
Cysteine is a semi-essential amino acid that is considered the rate-limiting amino acid in GSH synthesis. Cysteine’s donation of its sulfhydryl or thiol (-SH) group grants GSH its ability to function as an intracellular antioxidant. By itself, cysteine also functions as an extracellular antioxidant, serves as a precursor for taurine (necessary for magnesium metabolism), sulfate (necessary for detoxification), protein synthesis, and is a component of the enzyme acetyl-coenzyme A. Considering its diverse roles, cysteine insufficiency impacts a wide variety of functions. Because of its critical role in GSH production, cysteine deficiency limits the body’s ability to generate GSH and has been observed in conditions associated with oxidative stress.

Elevated cysteine levels may reflect blocks in one or more of its biochemical pathways, a result of genetic enzyme impairment or missing nutrient cofactors. Precise measurement of cysteine is thus valuable in identifying either deficiency or excess.

**Cystine and the Cysteine/Cystine Ratio**
In the process of being used as reducing agents, GSH and cysteine (Cys) are oxidized to their disulfide forms, glutathione disulfide (GSSG) and cystine (CySS), respectively. GSSG is typically reduced back to GSH via the enzyme glutathione reductase. Cystine is similarly restored to cysteine (with the help of glutathione and vitamin C, which can then be used to regenerate GSH). Because of this, measurement of the equilibrium between either the GSH <-> GSSG or Cys <-> CySS forms provides a reliable indicator of redox balance in the body.

A lower Cysteine/Cystine Ratio suggests a shift in redox balance in the direction of oxidative stress. Low ratios of Cysteine/Cystine (or GSH/GSSG) have been noted with aging, smoking, and chronic conditions such as diabetes, atherosclerosis, Parkinson’s disease, Alzheimer’s, amyotrophic lateral sclerosis (ALS), persistent atrial fibrillation, cataract formation, and cancer.

**Sulfate and the Cysteine/Sulfate Ratio**
Oxidation of the organic sulfur in cysteine generates inorganic sulfate, a critical factor for sulfation, a detoxification pathway responsible for the biotransformation of bile acids, steroid hormones, catecholamines, serotonin and phenols, as well as many xenobiotics and medications. Inorganic sulfate is also essential for mucin formation in the gastrointestinal tract and glycosaminoglycans in articular cartilage.
This conversion of cysteine to sulfate is termed ‘sulfoxidation.’ Compromise of any part of this biochemical process can lead to elevated levels of the intermediate sulfite, as well as deficient sulfate levels. Individuals who poorly convert cysteine to sulfate are more susceptible to environmental illness and disorders such as rheumatoid arthritis, Alzheimer’s, Parkinson’s disease, motor neuron disease, and even food sensitivities. High concentrations of sulfite have also been shown to directly lower TAC and increase lipid peroxides.

The Cysteine/Sulfate Ratio reflects the efficiency of this conversion, with high ratios suggesting sulfoxidation impairment, usually from inborn errors of metabolism or nutritional imbalances.

**ENZYME PROTECTION**

The body’s production of the potent antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) provides another important defense against oxidative damage. SOD catalyzes the conversion of the ubiquitous superoxide anion (O$_2^-$) to hydrogen peroxide (H$_2$O$_2$), which is then further inactivated to water by GPx.

**Superoxide Dismutase (SOD)**

SOD is critical for preventing the superoxide radical from generating other highly reactive species through its interactions with iron. These damaging molecules can initiate lipid peroxidation of fatty acids (a major cause of posttraumatic cell damage and death), form peroxyl radicals (implicated in colon cancer development in vitro), or react with nitric oxide to form another highly reactive compound, peroxynitrite (instrumental in the development of organ damage in circulatory shock). Because reduced SOD activity can result in the accumulation of intracellular superoxide, this SOD reaction constitutes a critical step in preventing oxidative stress. Imbalances in SOD have been noted in several disorders, such as familial ALS, Parkinson’s, Alzheimer’s, and other neurological diseases, Down’s syndrome, diabetes and impaired glucose tolerance, cataracts, and dengue fever.

SOD exists in two forms: cytosolic SOD, which uses zinc and copper as cofactors (CuZn-SOD), and mitochondrial SOD, which uses manganese as a cofactor (MnSOD). CuZn-SOD is widely distributed, comprising 90% of total SOD. The assay for SOD reflects both forms.

**Glutathione Peroxidase (GPx)**

GPx is a selenium-dependent enzyme found not only in the cytosol (70%), but also in the mitochondria (30%). Requiring four selenium atoms per active molecule, GPx scavenges lipid peroxides through cell membranes and quenches H$_2$O$_2$, the product of SOD, converting it to water. H$_2$O$_2$ is a weak and relatively stable oxidant, at least in the absence of iron and copper. However, in their presence, H$_2$O$_2$ can convert to the highly reactive hydroxyl radical. As a result, GPx is irreplaceable in the antioxidant arsenal, especially in the mitochondria, which do not contain catalase for protection from peroxide. GPx also offers exclusive protection from organic hydroperoxides (implicated in changes of vascular wall lipids that promote atherogenesis), and helps regenerate reduced vitamin C.

Imbalances in GPx have been observed with aging and a variety of disorders such as cancer, cardiovascular disease, diabetes, Alzheimer’s, alcohol-induced oxidative stress, cholecystitis, and urticaria.

**DAMAGE**

**Lipid Peroxides**

As discussed, oxidative stress results when pro-oxidants are insufficiently balanced by antioxidants, resulting in cellular damage. It is thus possible for antioxidant levels to be normal yet still inadequate in the face of excessive ROS production. Lipid peroxides are a direct marker of oxidative damage to polyunsaturated fatty acids (PUFAs).

Examples of lipid-rich sites in the body include the nervous system, lipoproteins such as LDL, and cell membranes. Lipid peroxidation is increased in many disease states as well as in tissues poisoned by toxins. Damaged tissues undergo lipid peroxidation more easily than healthy ones; thus, measurement of lipid peroxides is not only an excellent marker for oxidative damage but also tissue damage.

**8-Hydroxy deoxy-Guanosine (8-OHdG)**

Oxidative damage of DNA has been implicated as a fundamental cause of the physiologic changes and degenerative diseases associated with aging. When DNA is impacted by oxidative stress, 8-hydroxy-2’-deoxyguanosine (8-OHdG) is produced. This marker of oxidative stress has been shown to be significantly more elevated in studies of patients with heart failure and with breast and colorectal cancers compared to healthy controls.

Studies have shown diets rich in polyunsaturated fatty acids (like corn oil) induced DNA damage in a dose-dependent matter compared to diets rich in monounsaturated oils like olive oil. Vitamin C and olive oil may have a tempering effect on the generation of 8-OHdG.
CONCLUSION

Free radicals are introduced to the system from the environment and formed regularly in the course of normal metabolic processes. Inadequate antioxidant protection allows these ROS to alter cellular physiology, contributing to loss of function and the development of most chronic diseases. Oxidative stress is a lethal process that can persist long before appearance of symptoms. Evaluating redox balance can be done in a comprehensive fashion. Measurements of antioxidant reserve (GSH, cysteine and its metabolites, and TAC); enzyme function (SOD, GPx); and cellular damage (lipid peroxides and 8-OHdG) all provide not only a critical step in early identification of oxidative stress, but more importantly, a clinical opportunity to intervene and restore optimal redox balance.

References