Oxidative stress in human disease: Role of antioxidants

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Abstract

Damage to cells caused by free radicals is believed to play a central role in the aging process and in disease progression. Antioxidants are our first line of defense against free radical damage and are critical for maintaining optimum health and well being. The need for antioxidants becomes even more critical with increased exposure to free radicals. Pollution, cigarette smoke, drugs, illness, stress, and even exercise can increase free radical exposure. Because so many factors can contribute to oxidative stress, individual assessment of susceptibility becomes important. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. Many experts believe that the Recommended Dietary Allowance (RDA) for specific antioxidants may be inadequate and, in some instances, the need may be several times the RDA. As part of a healthy lifestyle and a well-balanced, wholesome diet, antioxidant supplementation is now being recognized as an important means of improving free radical protection.

Introduction

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Free radicals are capable of attacking healthy cells of the body, causing them to lose their structure and function.

Oxidative stress is defined as excessive production of reactive oxygen species (ROS) in the face of diminished antioxidant substances. The most common Reactive Oxygen Species include; the superoxide anion (O_2• -), the hydroxyl radical (OH•), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) (Halliwell and Gutteridge, 1985).

Free radical formation reactions

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad (1)$$

$$O_2^- + H_2O_2 \rightarrow OH^{\cdot} + OH^- \quad (2)$$

$$Fe^{3+} + O_2^- \rightarrow O_2 + Fe^{2+} \quad (3)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot} \quad (4)$$

Reactive free radicals play havoc on many biological systems. These are unstable chemical species. Although free radicals are essentials in our body metabolism to fight bacterial and viral infections, they can be harmful if present in excess leading to oxidative stress. They can induce oxidative damage to DNA, proteins, lipids which opens doors to various diseases like aging, Diabetes mellitus, inflammatory disorders, AIDS, cataract and also to neuro-degenerative diseases such as Alzheimer's and Parkinson disease.

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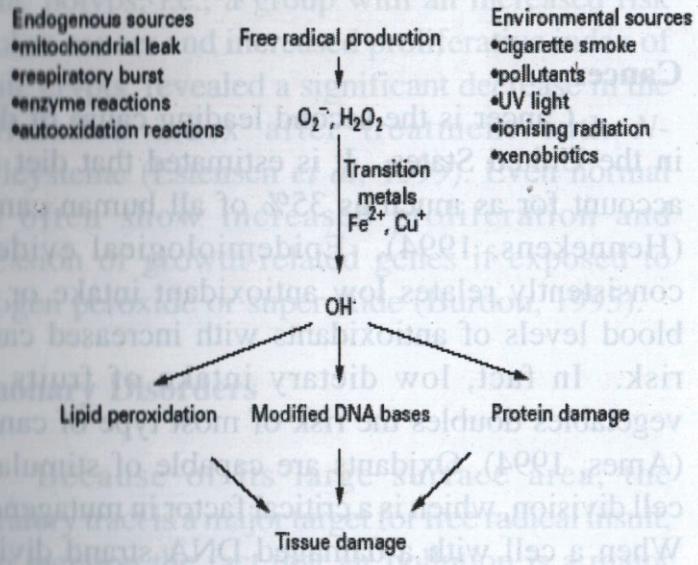
Major sources of free radicals in the body and consequences of free radical damage



Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline and brain dysfunction. Overall free radicals have been implicated in the pathogenesis of at least 50 diseases (Langseth, 1993; Halliwell, 1994). Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating. As remarkable as our antioxidant defense system is, it may not be always be adequate. The term "oxidative stress" has been coined to represent a shift towards the pro-oxidants in the pro-oxidant/ antioxidant balance that can occur as a result of an increase in oxidative metabolism. Increased oxidative stress at the cellular level can come about as a consequence of many factors, including exposure to alcohol, medications, trauma, cold, infections, poor diet, toxins, radiation, or strenuous physical activity. Protection against all of these processes is dependent upon the adequacy of various antioxidant substances that are derived either directly or indirectly from the diet. Consequently, an inadequate intake of antioxidant nutrient may compromise antioxidant potential, thus compounding overall oxidative stress.

Reactive oxygen species (ROS) is a term which encompasses all highly reactive, Oxygen-containing molecules, including free radicals. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage. ROS are generated by a number of pathways. Most of the oxidants are produced by cells occurs as:

- A consequence of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mitochondrial electron transport system.
- Oxidative burst from phagocytes (white blood cells) as part of the mechanism by which bacteria and viruses are killed, and by which foreign proteins (antigens) are denatured.
- Xenobiotic metabolism, i.e., detoxification of toxic substances.

Consequently, things like vigorous exercise, which accelerates cellular metabolism; chronic inflammation, infections, and other illnesses; exposure to allergens and the presence of "leaky gut" syndrome; and exposure to drugs or toxins such as cigarette smoke, pollution, pesticides, and insecticides may all contribute to an increase in the body's oxidant load.

Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction.

Oxidative stress as a frequent complication in disease conditions

Oxidative damage to DNA, proteins, and other macromolecules has been implicated in the pathogenesis of a wide variety of diseases, most notably heart disease and cancer (Halliwell, 1994).

There is a growing awareness that oxidative stress plays a role in various clinical conditions. Malignant diseases, diabetes, atherosclerosis, chronic inflammation, human immunodeficiency virus (HIV) infection, ischemia reperfusion injury, and sleep apnea are important examples. These diseases fall into two major categories. In the first category, diabetes mellitus and cancer show commonly a pro-oxidative shift in the systemic thiol/disulfide redox state and impaired glucose clearance, suggesting that skeletal muscle mitochondria may be the major site of elevated ROS production. These conditions may be referred to as "mitochondrial oxidative stress." Without therapeutic intervention these conditions lead to massive skeletal muscle wasting, reminiscent of aging-related wasting. The second category may be referred to as "inflammatory oxidative conditions" because it is typically associated with an excessive stimulation of NAD(P)H oxidase activity by cytokines or other agents. In this case increased ROS levels or changes in intracellular glutathione levels are often associated with pathological changes indicative of a dysregulation of signal cascades and/ or gene expression, exemplified by altered expression of cell adhesion molecules (Thiery *et al*, 1996).

Heart Disease

Heart disease is the leading cause of death in the United States. It is estimated that one in three Americans will eventually die from this disease (Hennekens and Gaziano, 1993). While several factors, such as high cholesterol levels, hypertension, cigarette smoking, and diabetes, are believed to promote atherosclerosis, a growing body of evidence suggests a critical step in its development is the oxidation of low-density lipoprotein (LDL) within the arterial wall (Jialal and Fuller, 1993). This theory is supported by several epidemiological studies which link low intakes of dietary antioxidants to an increased frequency of heart disease (Hennekens and Gaziano, 1993).

Antioxidants have been shown to prevent LDL oxidation *in vitro* and retard the progression of atherosclerosis in animal models. Several human studies found supplemental vitamin E increased vitamin E levels in LDL, increased the resistance of LDL oxidation, and decreased the rate of LDL oxidation (Hennekens and Gaziano, 1993). It has been estimated that dietary increases in antioxidant vitamins may reduce the risk of heart disease by 20-30% (Hennekens and Gaziano, 1993).

Cancer

Cancer is the second leading cause of death in the United States. It is estimated that diet may account for as much as 35% of all human cancers (Hennekens, 1994). Epidemiological evidence consistently relates low antioxidant intake or low blood levels of antioxidants with increased cancer risk. In fact, low dietary intake of fruits and vegetables doubles the risk of most type of cancers (Ames, 1994). Oxidants are capable of stimulating cell division, which is a critical factor in mutagenesis. When a cell with a damaged DNA strand divides, cell metabolism and duplication becomes deranged. Thus, a mutation can arise which in turn is an important factor in carcinogenesis. It is believed that antioxidants exert their protective effect by decreasing abnormal increases in cell division. Both cigarette smoking and chronic inflammation – two of the major causes of cancer – have strong free radical components in their mechanisms of action. Some research has indicated that people who smoke tend to have lower antioxidant levels than nonsmokers and are at an increased risk for both cancer and cardiovascular disease. Well over 100 studies have reported that reduction in cancer risk is associated with a diet high in vitamin C.

As mentioned earlier, the amount of fruits and vegetables included in the diet appears to have a significant impact on cancer risk. Although antioxidant activity is believed to be responsible for much of the protection against tumorgenesis, additional anticancer activities have been observed from several plant-derived substances. Sulfur containing phytochemicals, such as the allyl sulfides found in the allium family (garlic, onions, and leeks), and isothyocyanates and sulphoraphane (cabbage, broccoli, and cauliflower) have been shown to inhibit various steps in tumor development in animal and *in vitro* studies. Indoles, also found in cruciferous vegetables, and terpenes, natural constituents of citrus oils, may also be protective.

ROS are potential carcinogens because they facilitate mutagenesis, tumor promotion, and progression (Ha *et al*, 2000). A placebo controlled clinical study of patients with previous adenomatous colonic polyps, i.e., a group with an increased risk for colon cancer and increased proliferative index of colonic crypts, revealed a significant decrease in the proliferative index after treatment with *N*-acetylcysteine (Estensen *et al*, 1999). Even normal cells often show increased proliferation and expression of growth-related genes if exposed to hydrogen peroxide or superoxide (Burdon, 1995).

Pulmonary Disorders

Because of its large surface area, the respiratory tract is a major target for free radical insult, not to mention the fact that air pollution is a major source of ROS (Bland, 1995). Recent studies suggest that free radicals may be involved in the development of pulmonary disorders such as asthma (Greene, 1995). Cellular damage caused by free radicals is thought to be partly responsible for the bronchial inflammation characteristic of this disease. It has been suggested that increasing antioxidant intake may help to reduce oxidant stress and help to prevent or minimize the development of asthmatic symptoms (Greene, 1995). Vitamin C, vitamin E, and beta carotene supplementation has been associated with improved pulmonary function (Hatch, 1995). Some evidence suggests glutathione, or possibly N-acetyl cysteine, which is a precursor to glutathione, may be helpful in protecting against pulmonary damage as well (Bland, 1995). Other major pathologies that may involve free radicals include neurological disorders and cataracts (Kehrer and Smith, 1994). Neural tissue may be particularly susceptible to oxidative damage because it receives a disproportionately large percentage of oxygen and it has a high concentration of polyunsaturated fatty acids which are highly prone to oxidation (Muller, 1994). Formation of cataracts is believed to involve damage to lens protein by free radicals, causing the lens to lose its transparency. Some evidence suggests that cataract progression might be slowed with regular consumption of supplemental antioxidants, in particular vitamin E, vitamin C, and the carotenoids (Jacques *et al*., 1994)

Diabetes Mellitus

Elevated ROS levels have also been implicated in diabetes mellitus. In this case oxidative stress is associated with a pro-oxidative shift of the glutathione redox state in the blood (De mattia *et al*, 1998). Hyperglycemia is a hallmark of both non-insulin-dependent (type 2) and insulin dependent diabetes mellitus (type 1). Elevated glucose levels are associated with increased production of ROS by several different mechanisms (Nishikawa *et al*, 2000). In cultured bovine aortic endothelial cells, hyperglycemia was shown to cause increased ROS production at the mitochondrial complex II (Nishikawa *et al*, 2000). Treatment with antioxidants ameliorates diabetic complications including the dysfunction of endothelial cells or increased platelet aggregation (Ido *et al*, 1997).

Atherosclerosis

Atherosclerosis is a multifactorial disease characterized by hardening and thickening of the arterial wall. The vascular areas affected by this disease contain mononuclear cells, proliferating smooth muscle cells, and extracellular matrix components. Atherosclerosis is commonly viewed as a chronic inflammatory disease and is associated with certain risk factors such as hyperlipidemia, diabetes and hypertension. Excessive ROS production has been implicated in the pathogenesis of atherosclerosis and hypertension (Slund *et al*, 1999) Oxidative stress induces the expression of protein kinases such as focal adhesion kinase and intercellular adhesion molecules such as ICAM-1 (Chien and Shyy, 1998). The invasion of the artery wall by monocytes and T lymphocytes is one of the earliest events in the development of atherosclerotic lesions. Monocytes, macrophages, and smooth muscle cells possess the so-called scavenger receptor for oxidized LDL. Binding of oxidized LDL leads to the activation of monocytes and macrophages and stimulates the expression of Mn-SOD which, in turn, increases the concentration of hydrogen peroxide by perturbing the steadystate levels of ROS. This process is associated with massive macrophage apoptosis and contributes

thereby to the formation of the atherosclerotic lesions (Kinscherf *et al*, 1999). The process may be further enhanced by cytokines and other factors such as TNF, interleukin-1b, angiotensin II, and interferon-g, which induce superoxide production by the membrane-bound NADPH oxidase in endothelial cells (De Keulenaer *et al*, 1998).

Neurodegenerative Diseases

Down's syndrome or trisomy 21 is the most frequent genetic cause of mental retardation and is commonly associated with the development of Alzheimer's disease (AD) in adult life. Cultured cortical neurons from fetal Down's syndrome cases exhibit a three- to-fourfold higher intracellular ROS level than age-matched normal brain cells. Treatment with free radical scavengers or catalase prevents the degeneration of Down's syndrome neurons in culture (Busciglio and Yankner, 1995).

Rheumatoid Arthritis

While the enhancement of immune reactivity by prooxidative conditions may be critically important for the immune system to control and defeat rapidly multiplying pathogens, such enhancement also bears the risk of inducing autoimmune processes. Rheumatoid arthritis is a systemic autoimmune disease characterized by chronic joint inflammation with infiltration of macrophages and activated T cells. Several lines of evidence suggest that production of free radicals at the site of inflammation may contribute decisively to the pathogenesis of this disease (Araujo *et al*, 1998). T cells isolated from the synovial fluid of patients with rheumatoid arthritis are characterized by a decreased intracellular GSH level and the "primed" CD45RO phenotype. These T cells exhibit severely impaired phosphorylation of the adaptor protein linker for T-cell activation (LAT). Changes in intracellular GSH level were shown to alter the subcellular localization of LAT (Gringhuis *et al*, 2000). The migration of monocytes and lymphocytes into the rheumatoid arthritis synovium is mediated by the abnormal expression of several adhesion molecules including ELAM-1, VCAM-1, ICAM-1, and ICAM-2, an effect which may be explained by the abnormal induction of redox-sensitive signaling pathways. Oxidative conditions in synovial tissue are also associated with a higher incidence of p53 mutations (Veale and Maple, 1996). Although malignant tumors of the synovium are rare, it has been hypothesized that the presence of transformed cells in the synovium of rheumatoid arthritis patients may lead to progressive joint destruction without malignant degeneration. The heat shock protein hsp65/60, which has been implicated in the pathogenesis of atherosclerosis, is also a candidate (auto) antigen in the pathogenesis of rheumatoid arthritis.

HIV Infection

HIV infection is associated with progressive deterioration of the immune system, leading eventually to lethal opportunistic infections. Relatively early in the course of HIV infection, there is a decrease in various functional activities of lymphoid cells followed by a conspicuous decrease in CD41 T-lymphocyte numbers. In the late stages of the disease, the patients often suffer from massive skeletal muscle wasting. HIV infection is also associated with massive catabolism of cysteine into sulfate. This process can be detected even in the early asymptomatic state of the disease and accounts for a mean net loss of more than 4 g cysteine/day. Excessive cysteine catabolism can be detected most easily as urinary sulfate excretion or as muscular sulfate excretion determined from arterial-venous differences in the lower extremities (Breitkreutz *et al*, 2000).

Numerous lymphocyte functions are exquisitely sensitive to a decrease in intracellular glutathione levels. That the virus induced cysteine deficiency is indeed a causative factor for the progressive impairment of the immune system is suggested by two independent placebo-controlled double blind studies, which demonstrated that treatment of HIV infected patients with *N*-acetylcysteine leads to a significant improvement of various proliferative T-cell responses and to a reconstitution of NK cell activity to almost normal levels. In view of the importance of glutathione levels

in several redox-regulated systems, it is believed that the HIV-induced decrease in intracellular glutathione levels facilitates the induction of signaling pathways leading to lymphocyte activation but renders the cells more sensitive to oxidative stress. Ex vivo labeling studies have shown that, compared with healthy controls, HIV-infected patients have indeed significant increases in the number and faction of dividing CD41 and CD81 T cells. The fact that CD41 T-cell counts decline during the course of HIV infection suggests, however, that the increase in CD41 T cell destruction is greater than the increase in T-cell production (Lempicki *et al*, 2000). Taken together, the available evidence suggests that depletion of the systemic cysteine pool may be one of several ways by which a virus can prevent its elimination by the immune system. Because immune reconstitution is a widely accepted aim of HIV therapy, cysteine supplementation may be considered as a standard therapy for these patients.

The Mitochondria and Oxidative stress

The mitochondria are the energy power houses of the cell. Due to their critical role in producing the energy that derives every physiologic process, mitochondrial function is an area of intense interest and study today. It has been suggested that certain chronic illnesses related to muscle pain and chronic fatigue, e.g., myofascial pain syndrome (MPS), fibromyalgia syndrome, and chronic fatigue immunodeficiency syndrome (CFIDS), are disorders in which there is an aberration or dysfunction of mitochondrial energy production. It has been suggested that mitochondrial dysfunction is related to damage caused by ROS produced as a consequence of increased oxidative stress and insufficient antioxidant defenses (Shigenaga and Ames, 1994). Levels of ROS produced within the mitochondria are reported to increase with age. Consequently, oxidative damage to mitochondria would also appear to increase with age. This damage results in a decrease in energy production by some of the cell's mitochondria.

Antioxidant protection

To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals (Jacob, 1995). These components include:

- Nutrient-derived antioxidants like ascorbic acid, tocopherols and tocotrienols, carotenoids, and other low molecular weight compounds such as glutathione and lipoic acid
- Antioxidant enzymes, e.g., superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions.
- Metal binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions.
- Numerous other antioxidant phytonutrients present in a wide variety of plant foods.

Various Reactive Oxygen Species and corresponding neutralizing antioxidants

ROS	Neutralizing Antioxidants
Hydroxyl radical	Vitamin C, glutathione, flavanoids, lipoic acid
Superoxide radical	Vitamin C, glutathione, flavanoids, SOD
Hydrogen peroxide	Vitamin C, glutathione, beta carotene, vitamin E, CoQ_{10}, flavanoids, lipoic acid
Lipid peroxides	beta carotene, vitamin E, ubiquinone, flavanoids, glutathione peroxidase

Dietary Antioxidants

Vitamin C, vitamin E, and beta carotene are among the most widely studied dietary antioxidants. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids. It is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Vitamin c has been cited as being capable of regenerating vitamin E. Beta carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues. Research suggests beta carotene may work synergistically with vitamin E (Jacob, 1995). A diet that is excessively low in fat may negatively affect beta carotene and vitamin E absorption, as well as other fat-soluble nutrients. Fruits and vegetables are major sources of vitamin C and carotenoids, while whole grains and high quality, properly extracted and protected vegetable oils are major sources of vitamin E.

Endogenous Antioxidants

In addition to dietary antioxidants, the body relies on several endogenous defense mechanisms to help protect against free radical-induced cell damage. The antioxidant enzymes – glutathione Peroxidase catalase, and superoxide dismutase (SOD) – metabolize oxidative toxic intermediates and require micronutrient cofactors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity. It has been suggested that an adequate dietary intake of these trace minerals may compromise the effectiveness of these antioxidant defense mechanisms. Research indicates that consumption and absorption of these important trace minerals may decrease with aging (Duthie and Brown, 1994).

Lipoic acid, yet another important endogenous antioxidant, categorized as a "thiol" or "biothiol," is a sulfur-containing molecule that is known for its involvement in the reaction that catalyzes the oxidative decarboxylation of alpha-keto acids, such as pyruvate and alpha-ketoglutarate, in the Krebs cycle. Lipoic acid and its reduced form, dihydrolipoic acid (DHLA), are capable of quenching free radicals in both lipid and aqueous domains and as such has been called a "universal antioxidant." Lipoic acid may also exert its antioxidant effect by chelating with pro-oxidant metals. Research further suggests that lipoic acid has a sparing effect on other antioxidants. Animal studies have demonstrated supplemental lipoic acid to protect against the symptoms of vitamin E or vitamin C deficiency (Packer and Witt, 1995).

The importance of balance

Although much of the research to date focuses on the potential benefit of single antioxidant nutrients, it has become clear that the best protection against oxidative stress comes from a wide assortment of interrelated antioxidants and antioxidant cofactors (Jacob, 1995). In other words, the human body utilizes an integrated antioxidant system composed of several players that work together as a team. The reducing potential of each individual member of the antioxidant defense team is enhanced when a full complement of players is available. For example, some evidence suggests a poor concentration of any single one of the antioxidants vitamin C, vitamin E, or beta carotene, appears to increase the risk of cardiovascular disease. Additionally, the combination of several suboptimal concentrations may have an peroxiadditive or even synergistic affect on increasing risk.

Conversely, it has been suggested that, under certain conditions, an excess of any type of antioxidant in the absence of balance with the others may actually be counter-protective (Bland, 1995). Moreover, the relative importance of a given antioxidant may vary with different disease conditions because the other type or types of ROS generated are likely to differ, and because varying levels of specific antioxidants exist within the different tissues of the body.

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