Role of Antioxidants in Health Maintenance

VISHWANATH M. SARDESAI, PHD
Department of Biochemistry and Surgery, Wayne State University, Detroit

ABSTRACT: Free radicals are produced in the body as by products of normal metabolism and as a result of exposure to radiation and some environmental pollutants. Because they are highly reactive, they can damage cellular components and are implicated in a variety of diseases. Free radicals are normally neutralized by efficient systems in the body that include the antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) and the nutrient-derived antioxidant small molecules (vitamin E, vitamin C, carotenes, flavonoids, glutathione, uric acid, and taurine). In healthy individuals, a delicate balance exists between free radicals and antioxidants. In some pathologic conditions such as diabetes, and in critically ill patients, oxidative stress causes the level of antioxidants to fall below normal. Antioxidant supplements for such conditions are expected to be of benefit. As a preventive measure against certain diseases, the best approach for healthy individuals is to regularly consume adequate amounts of antioxidant-rich foods, eg, fruits and vegetables.

ANTIOXIDANTS AND HEALTH

The relationship between diet and some chronic diseases in humans has been the topic of investigation for a number of years. Recently epidemiologic and experimental data have suggested a benificial role of antioxidants present in food. The basis for this favorable effect incorporates the theory that antioxidants effectively neutralize free radicals, which can damage cells and initiate disease, and thus can offer protection from many types of diseases. In addition to their presence in food sources, antioxidant supplements are readily available on the open market, and people are consuming them in mega-quantities as a preventive measure. In this brief review, the formation of free radicals and the rationale for the potential health benefits of antioxidants in food are discussed.

Free Radicals. A free radical is defined as a chemical species possessing an unpaired electron in its outer orbit.[1] It is this unpaired electron that makes the free radical unstable and, therefore, quite reactive because it tends to react with other molecules in order to pair this electron and generate a more stable species. The most important free radicals in biologic systems are derivatives of oxygen.

Reactive oxygen radicals arise as byproducts of normal metabolism as well as by exposure to sunlight, ozone, radiation, tobacco smoke, and other environmental pollutants. One of the commonly generated free radicals is superoxide anion. A major source of superoxide is the activity of mitochondrial and microsomal electron transport chains. Xanthine oxidase and aldehyde oxidase-catalyzed reactions produce superoxide radical by adding a single electron to molecular oxygen. A number of heavy metals that are normally present in the cells and tissues can accept an electron from free radicals or biologic sources and transfer it to molecular oxygen to form superoxide anion radical. Superoxide anion can gain an additional electron from the electron transfer chain or at random from an organic or inorganic species in its immediate vicinity. The product formed is the peroxyl anion, which can form hydrogen peroxide with two protons from solution. Hydrogen peroxide does not qualify as a radical because it contains no unpaired electron.

Divalent cations such as iron and possibly copper may cause catalyzed interaction of superoxide and hydrogen peroxide that forms the hydroxyl radical, which is highly reactive and potentially more damaging than superoxide in living systems. It can abstract an electron from almost any organic mol-

Correspondence and Reprint Requests: V. M. Sardesai, PhD, Department of Surgery, Wayne State University Health Center, 4201 St. Antoine 6-C, Detroit, MI 48201.

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ecule in its vicinity, including DNA (causing strand breakage and base modification) and proteins, which can initiate additional radical formation and biochemical changes that can lead to disease. The generation of most common free radicals is presented in Figure 1.

An additional reactive metabolite of oxygen is singlet oxygen, which can be formed as an excited state of oxygen by energy capture. A peripheral electron in the oxygen structure is excited to an orbital above that which it normally accompanies. It is not a free radical, but it may be of significance in certain biochemical processes such as tissue damage.

Free radicals can cause many adverse reactions in vivo that result in cell injury or dysfunction and subsequent inflammation and degenerative disease states.[2] Because they are so highly reactive, free radicals generally react with the first molecule that they encounter, most frequently the lipid component of cell or organelle membranes. A single hydroxyl radical and molecular oxygen can react with a polyunsaturated fatty acid and alter its structure or functional integrity. This altered polyunsaturated fatty acid generates multiple fatty acid peroxy radicals that react with other lipids, proteins, and nucleic acids, and thereby propagate a chain reaction involving electron transfer. Fatty acid peroxide molecules break up and form dialdehydes, such as malonaldehyde, which can cause cross-linking between various types of molecules that leads to cytotoxicity, mutagenicity, membrane breakdown, and enzyme modification. Malonaldehyde also polymerizes with itself and other tissue breakdown products to form an insoluble pigment lipofuscin, which accumulates in some aging tissues.[3] Singlet oxygen, hydrogen peroxide, and the oxygen free radicals are referred to as reactive oxygen species.

Defense Against Free Radicals. The human body is under constant assault by reactive oxygen molecules. Free radicals form in almost every cell of the body at an astounding rate. Because reactive oxygen species can cause damage to cells and tissues, our bodies have evolved various antioxidant defense mechanisms that combat their constant barrage. Overwhelming evidence now indicates that antioxidants play a critical role in wellness, health maintenance, and the prevention of chronic and degenerative diseases.

There are two types of antioxidants in the body: (1) antioxidant enzymes, which prevent the generation of toxic substances, and (2) antioxidant small molecules, which intercept any free radicals and singlet oxygen that are generated. These antioxidants exist in both the membranes and the aqueous compartments of cells.

Enzymes. The enzyme superoxide dismutase (SOD) is the first line of defense against oxygen

GENERATION OF FREE RADICALS

a) $O_2 + (e^-) \longrightarrow O_2^-$ (superoxide radical)

b) $Fe^{+2} + O_2 \longrightarrow Fe^{+3} + O_2^-$ (superoxide radical)

c) $O_2^- + (e^-) + 2H^+ \longrightarrow H_2O_2$

d) $O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$

e) $H_2O_2 + (e^-) + 2H^+ \longrightarrow H_2O + OH^\bullet$ (hydroxyl radical)

f) $Fe^{+2} + H_2O_2 \longrightarrow Fe^{+3} + OH^- + OH^\bullet$ (hydroxyl radical)

Figure 1. Generation of free radicals. a) Addition of an electron to oxygen forms superoxide anion radical; b) in the presence of oxygen, ferrous iron is oxidized to ferric iron, and oxygen is converted to superoxide; c) superoxide in the presence of electron and hydrogen ions can form hydrogen peroxide nonenzymatically or d) superoxide can react with hydrogen ions and form hydrogen peroxide (catalyzed by superoxide dismutase; e) hydrogen peroxide either in the presence of an electron and hydrogen ion or f) in the presence of ferrous iron can give rise to the more toxic hydroxyl radical.

toxicity.[4] Superoxide is not membrane-permeable, and it can accumulate in the cellular fraction where it is produced. Three forms of SOD are present in humans to prevent this accumulation: a cytosolic copper/zinc SOD-1, a mitochondrial manganese-dependent SOD-2, and an extra-cellular copper/zinc SOD-3. The enzyme catalyzes the conversion of superoxide to hydrogen peroxide, which is less toxic to tissues than superoxide. Hydrogen peroxide is metabolized by glutathione peroxidase, which is a selenium-containing enzyme present in the cytosol and mitochondria or catalase located in the peroxisome organelles (into which hydrogen peroxide would diffuse). Catalase is a heme-containing enzyme that utilizes copper as a metal cofactor; it is specific for hydrogen peroxide.

Glutathione peroxidase requires reduced glutathione as the source of reduced equivalents. In addition to its action on hydrogen peroxide, glutathione peroxidase has broader substrate specificity. It has the ability to use complex peroxides as substrates, including toxic lipid peroxides, and to convert them to corresponding inert alcohols.

The activities of antioxidant enzymes depend on the nutritional availability of the so-called antioxidant minerals: manganese, copper, zinc, and selenium. Any deficiency of these nutrients may lower the levels of antioxidant enzymes and initiate disease processes. Little evidence exists regarding low

levels of dietary intake of manganese or zinc as risk factors. Major disturbances of antioxidant status such as low SOD occur in copper deficiency in rats and increase in the generation of aortic lipid peroxides.[5] Selenium may also prove to be of major significance; it is usually present in adequate amounts in the diet. The dietary level of this nutrient is low in certain parts of the country, and foods raised in these regions have low levels of this mineral. This effect is minimized by the current food distribution system, which assures that foods marketed in any one area are derived from a number of different geographic locations.

Nonenzymatic Antioxidants. The highly efficient antioxidant enzymes function in the cell to keep the reactive oxygen-derived molecular species at a minimum. However, enzymatic defenses against certain types of reactive oxygen species (eg, singlet oxygen and hydroxyl radical) are either ineffective or totally lacking. The body must rely on food-derived substances with antioxidant character to effectively neutralize such species. These antioxidants serve as scavengers that mop up the hydrophobic membrane, the hydrophilic cytosol, and sometimes the extracellular compartment; they include vitamins E and C, carotenes, glutathione, uric acid, taurine, and flavonoids.

Vitamin E, a series of isomers of tocopherol, is lipid soluble and is thought to be the most important antioxidant found in lipid membranes in the body.[6] It protects the polyunsaturated fatty acids in the membranes against peroxidation by scavenging peroxy radicals. Tocopherols are widely distributed in nature, and the richest sources are vegetable oils.

Vitamin C is the most abundant water-soluble antioxidant in the body.[1] It directly reduces free radicals with the concurrent formation of dehydroascorbate. Vitamin C can also regenerate vitamin E from the tocopherol radical. Fruits, especially citrus varieties and berries, tomatoes, lettuce, and green peppers are excellent sources.

Carotenes are a set of several hundred fat-soluble pigments present in yellow and green fruits and vegetables. In addition to being precursors of vitamin A, these pigments are also excellent antioxidants and radical-trapping agents, especially for peroxyl and hydroxyl radicals.[7,8] Carotenes serve as another dietary source of lipid-soluble antioxidants, which are important in protecting lipid membranes against oxidation.

Flavonoids are a large group of polyphenolic antioxidants that occur naturally in fruits and vegetables and in beverages such as tea and wine.[9] The most important flavonoids are anthocyanins, flavonols, and flavones. Flavonoids are water-soluble scavengers of singlet oxygen and of superoxide, peroxyl, and lipid peroxyl radicals.[10,11]

In addition to the dietary antioxidants, there are endogenous substances that serve as important radical scavengers. Glutathione is a tripeptide synthesized intracellularly by mammalian cells. Its concentration varies as a function of dietary sulfur-containing amino acids.[12] It is a substrate for glutathione peroxidase, which is involved in detoxification of hydrogen peroxide and lipid hydroperoxides. It can also scavenge singlet oxygen and superoxide and hydroxyl radicals.[13] Although primarily considered the waste product of purine metabolism, uric acid shows strong antioxidant activity. It is a circulating water-soluble free radical scavenger, it interacts directly with hydroxyl radical,[14] and it protects red cells from peroxidation lysis. Uric acid levels in blood are directly related to dietary intake of purines. However, excessive uric acid concentrations can cause gout. Humans have higher uric acid in blood than other mammals do, and it has been suggested that this antioxidant contributes to the prolonged life span of humans.[15]

The amino acid taurine is an effective free radical scavenger and normally is concentrated in cells and tissues that possess considerable potential for producing oxidants. For example, there is a high concentration of taurine in the retina, a tissue in which various oxidants are generated photolytically and enzymatically,[16] and in neutrophils, which enzymatically produce oxidants during the phagocytic process.[17] There is no evidence that taurine levels can be increased by dietary means.

Free Radicals and Disease States. Free radicals play a role in a wide variety of pathologic conditions and may also be important in the aging process.[18] They react with almost any component in the cell and contribute to many types of diseases. If their target is DNA, the likelihood of cancer increases; if their target is low-density lipoprotein (LDL) in blood, arteriosclerosis may result. In conditions such as diabetes, an increase in oxidants causes a decrease in blood antioxidants such as vitamin C. Cigarette smokers exhibit an elevated oxidant status and lower levels of both circulating carotenes and vitamin C. It is estimated that vitamin C intake for smokers should be at least 200 mg/d to provide the same degree of protection against hypovitaminosis C as that provided by 60 mg/d in nonsmokers.[19] In critically ill patients, oxidative stress is a major problem, and the circulating concentration of vitamin E falls.[20] Because there may be a defect in the absorption of lipid-soluble vitamins in some patients, it may be advantageous to supply water-miscible emulsions or to administer them intramuscularly.

Some selected diseases associated with free radicals are discussed below.

Cardiovascular Disease. The role of free radicals in the cause of cardiovascular disease has been a topic of considerable interest and attention. High levels of plasma cholesterol, particularly LDL cho-

lesterol, is one of the risk factors linked to the development of atherosclerosis. Data from several studies suggest that LDL is oxidatively modified by free radical reactions and that this oxidized low density lipoprotein plays an important role in the initiation of the atherogenic process, allowing its infiltration into the vessel wall.[21] Patients with cardiovascular disease have higher than normal serum levels of lipid peroxides, and oxidized lipids are frequently seen in atherosclerotic lesions. Indeed, Salonen et al[22] showed an association between oxidatively modified LDL and the progression of atherosclerosis. High iron stores are associated with excess risk of myocardial infarction,[23] which is attributed to the role of iron in increasing the generation of hydroxyl radicals. Indian immigrants in the United Kingdom who consume high amounts of clarified butter tend to have higher than expected mortality from cardiovascular disease despite the absence of obvious risk factors.[24] Clarified butter contains large amounts of cholesterol oxides whereas natural butter contains only trace amounts. The cholesterol oxides are assumed to be incorporated in LDL and may be responsible for high mortality rates among Indian immigrants.

If LDL oxidation is the initial event in atherosclerotic processes, the antioxidant status of individuals should have a major effect on the rate of LDL and the development of atherosclerosis. Epidemiologic studies show a strong inverse correlation between plasma antioxidant status and risk of cardiovascular disease, and the most significant factor is vitamin E.[25] The number of deaths attributed to coronary disease was reduced by 40% in subjects who consumed 100 IU of vitamin E per day (the recommended dietary allowance is 15 IU) compared with those whose dietary intake of this vitamin was low. Dietary carotenoids are also inversely related to heart disease and stroke in the Physicians Health Trial,[26] and plasma beta carotene concentrations are inversely related to the risk of angina.

Recent studies have demonstrated that vitamin C is more potent in preventing LDL oxidation than is vitamin E, and the combination of the two vitamins is even more effective than either one alone. Plasma levels of vitamins C and E are higher in European populations with low incidences of cardiovascular disease.[27]

In most countries, a high dietary intake of fat and the level of serum cholesterol are strongly correlated with mortality from coronary heart disease. However, in certain regions of France this relationship is less apparent. Mortality from coronary heart disease is lower in France than in the United States or the United Kingdom despite the fact that individuals in all three countries have similar ranges of serum cholesterol levels. Moreover this French paradox cannot be explained by differences in other risk factors for heart disease. In France there is regular consumption of moderate amounts of wine and other alcoholic beverages. It is known that alcohol causes an increase in high density lipoprotein cholesterol, which is considered antiatherogenic.[28] Red wine is also rich in flavonoids, which are more potent as antioxidants than vitamin E is, and it is suggested that these compounds contribute substantially to the reduction of mortality from coronary heart disease in France.[29]

The beneficial effects of antioxidants have been studied experimentally in rabbits, and the results suggest that protecting LDL from oxidation slows the progression of the atherosclerotic process. Probucol is a drug that reduces plasma cholesterol and also serves as an antioxidant. It is carried by LDL and essentially prevents its oxidation. In hypercholesterolemic rabbits, probucol retarded the progression of atherosclerotic lesions by 50% compared with those in a control group that had cholesterol levels lowered to comparable levels with lovastatin, which is not an antioxidant.[30]

Cancer. Oxygen free radicals are capable of oxidatively modifying DNA and may be the central cause of some cancers. However, a direct connection between oxidatively modified DNA and cancer is not available because of the limited understanding of carcinogenic mechanisms and because no single factor can explain the entire process. But there is extensive indirect evidence supporting a role of free radicals in carcinogenesis. In the last two decades, many epidemiologic and clinical studies as well as investigations in experimental systems have provided evidence suggesting a role of free radicals in the cause of cancer, particularly of epithelial cell origin. The intake of metal such as iron, which facilitates the production of active oxygen species, is correlated with cancer development in humans and animals.[31] There is evidence that hepatocellular carcinoma is related to severe iron overload in patients with homozygous hereditary hemochromatosis, and recent epidemiologic studies point to the possibility of an association between moderately increased body iron stores and an increased risk of cancer in the general population.[32] The free radical scavengers vitamins C and E and beta carotene have been shown to protect against cancer development in animal models and seem to be promising candidates for preventing cancer in humans.[33]

Dietary beta carotene is associated with decreased risk of several types of cancer, including cervical cancer. However, the most consistent association is that of decreased dietary beta carotene or its low plasma level with increased risk of lung cancer.[34,35] The use of vitamin E supplements also offers protection against this type of cancer. Breast cancer is less consistently related to carotene than is lung cancer, but it is inversely related to the intake of vitamin C.[35] Stomach cancer is also associated with low intake of vitamin C. Colorectal cancer risk is lowest in popu-

lations whose diets typically are rich in fruits and vegetables (sources of antioxidants).[36]

Animal studies showed that the antioxidant mineral selenium (usually at high levels of intake) protects against a variety of cancers.[37]

Brain. The brain and central nervous system seem to be particularly susceptible to free–radical-mediated damage. Polyunsaturated fatty acids, substrates for lipid peroxides, are major constituents of cell membranes, and in addition some brain cells contain higher concentrations of iron, which promotes cytotoxic radical formation.

Amyotrophic lateral sclerosis is a progressive degenerative disorder of motor neurons in the spinal cord, brain stem, and motor cortex characterized by varying degrees of weakness, atrophy, fasciculation, and spasticity. It has recently been established that this disease is linked to defects in the SOD^1 gene, which encodes the cytosolic SOD.[38] Individuals with this disease apparently are unable to get rid of superoxide efficiently. Free radicals seem to contribute to the pathogenesis of other neurodegenerative disorders such as Parkinson's disease, in which there is substantial evidence of oxidative stress.[39] Moreover the postmortem brain iron content of those with Parkinson's disease shows a 176% increase in total iron in substantia nigra. Oxidative tissue degeneration also seems to be a key feature in Alzheimer's disease, Down's syndrome, and other neurologic disorders.[40]

Reperfusion Injury. Temporary interruption of blood to tissues causes damage to those tissues and is assumed to occur as a result of depletion of adenosine triphosphate during the period of hypoxia. If the duration of ischemia does not do irreversible damage, tissue function can be salvaged by reperfusion. However, reperfusion during the termination of ischemia can also cause additional damage to hypoxic cells, a phenomenon that has been termed "reperfusion injury." Although other factors may be involved,[41] most researchers agree that free radicals play a major role in the pathogenesis of reperfusion injury.

During the first 30 to 60 seconds of postischemic reperfusion, there is generation of superoxide, which can combine with iron released by hypoxic cells to form hydroxyl radical, the initiator of lipid peroxidation.[42] Studies in isolated organs showed that tissue function is better preserved if antioxidants are incorporated in the reoxygenation medium at the end of the ischemic period. Inhibition of lipid peroxidation after ischemia and reperfusion accordingly protects tissues such as the heart.[43]

Cataracts. Cataracts are a condition in which opaque regions develop within the normally transparent lens of the eye, resulting in significant loss of visual acuity. It is one of the major causes of age-dependent visual impairment and blindness. Although the exact cause of this disease is unknown, intraocular generation of oxygen radicals may constitute one of the significant risk factors in cataractogenesis. Hydrogen peroxide is present (20 to 30 μmol/L) in the aqueous humor and can give rise to hydroxyl radicals. Epidemiologic and experimental studies suggest that antioxidants may be prophylactically or therapeutically useful against this disease.[44] Vitamin C is of particular interest because of the high levels of ascorbic acid (30-fold to 35-fold over plasma level) in the human lens. There is reduced risk of senile cataracts with an increased intake of vitamin C as well as of other antioxidants such as beta carotenes and vitamin E.[45]

Beneficial Effects of Free Radicals. Free radicals participate in a number of metabolic processes essential for life. Some enzymes utilize superoxide as a substrate for their normal catalytic activity. Metabolism of arachidonic acid by cyclooxygenase to form prostaglandins is regulated by free radical reactions at certain sequential steps. One good example of the benefits of free radical production is the production and mobilization of oxygen free radicals by neutrophil granules, which are involved in body defense. Neutrophil granules produce and mobilize oxygen free radicals to destroy the bacteria and other cells or foreign matter that neutrophil granules ingest.

The membrane surface of neutrophils contains an NADPH-dependent oxidase system that is a highly efficient source of superoxide radical generation. The enzyme is normally dormant, but after phagocytosis occurs, the oxidase becomes active, resulting in the production of superoxide. This is accompanied by considerable consumption of oxygen by the cells, a phenomenon termed "respiratory burst," and it accounts for more than 90% of the oxygen consumption by stimulated cells. The superoxide is the precursor of hydrogen peroxide, which gives rise to hypochlorous acid (common in laundry bleach) in the presence of chloride ions and myeloperoxidase (another enzyme). It is a powerful oxidizing agent responsible for much of the neutrophil toxicity that kills bacteria. Myeloperoxidase is unique to and abundant within neutrophils.

The genetic inability of neutrophils to produce superoxide (absence of oxidase) causes the life-threatening condition known as chronic granulomatous disease. Neutrophils from such individuals have seriously impaired ability to kill microorganisms that have been ingested, leading to multiple recurrent local infections and often septicemia and death at an early age.[46]

Excess Intake of Antioxidants. We tend to view oxidants as bad and antioxidants as good. There is certainly a dietary need for antioxidants to neutralize the oxidants formed endogenously, but the optimum amounts needed by humans are yet to be determined. We also do not have data showing that long-term (life time) intake of large doses of antioxidants will not be toxic.[47] Some *in vitro* tests suggest

the possibility that high doses of a single antioxidant in the presence of low levels of other micronutrients and a high oxidative state may result in cell injury.[48] Data published recently on clinical trials indicate that antioxidant supplements do not reduce the incidence of tumors. In fact, rates of lung cancer and number of deaths from heart disease increase with antioxidant supplements compared with rates and number of deaths in the group receiving placebo.[49] Treatment for 4 years with either beta carotene or vitamin C does not seem to affect the rate of occurrence of adenomas in patients who had adenomas removed before entering the study.[50] The lack of benefits with antioxidant supplements and the possible deleterious effects reported in these studies seem to conflict with the reduced risk suggested by epidemiologic investigation and short-term experiments.

The ingestion of excessive amounts of antioxidant is presumed to shift the oxidant-antioxidant balance toward the antioxidant side and to prevent potential damage by oxidants. However, excessive antioxidants may adversely affect key physiologic processes that are dependent on free radicals and also may interfere with the defense mechanism of neutrophils. There is a need to study the long-term (several years) effect of consuming large doses of a single antioxidant in humans.

CONCLUSION

The human body is under constant assault by oxygen free radicals produced as a consequence of normal biochemical activity. Because the damage caused by free radicals can be life-threatening, the body has many overlapping defense mechanisms to protect against oxidants. These include the antioxidant enzymes and several nutrient-derived antioxidant small molecules.

A delicate balance is maintained between oxidants and antioxidants in healthy individuals. In some pathologic conditions, the balance may be tilted toward the oxidant state. Antioxidant supplements seem to reduce the incidence of some diseases, but the possibility remains that excessive levels of antioxidants can be toxic (this applies to all nutrients). The best preventive measure against certain diseases is to regularly consume antioxidant-rich foods, fruits and vegetables.

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