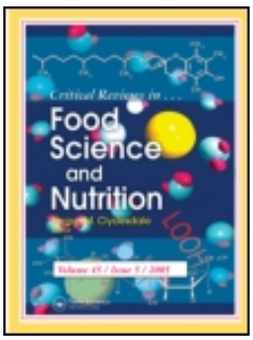

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

Antioxidants and Prevention of Chronic Disease

JOYE K. WILLCOX , SARAH L. ASH & GEORGE L. CATIGNANI

To cite this article: JOYE K. WILLCOX , SARAH L. ASH & GEORGE L. CATIGNANI (2004) Antioxidants and Prevention of Chronic Disease, Critical Reviews in Food Science and Nutrition, 44:4, 275-295, DOI: 10.1080/10408690490468489

To link to this article: https://doi.org/10.1080/10408690490468489

Published online: 10 Aug 2010.

Critical Reviews in Food Science and Nutrition, 44:275–295 (2004)

ISSN: 1040-8398
DOI: 10.1080/10408690490468489

Taylor & Francis
Taylor & Francis Group

Antioxidants and Prevention of Chronic Disease

JOYE K. WILLCOX
Department of Food Science, North Carolina State University, Raleigh, North Carolina, USA

SARAH L. ASH
Department of Animal Science, North Carolina State University, Raleigh, North Carolina, USA

GEORGE L. CATIGNANI
Department of Food Science, North Carolina State University, Raleigh, North Carolina, USA

The generation of reactive oxygen species (ROS) and other free radicals (R●) during metabolism is a necessary and normal process that ideally is compensated for by an elaborate endogenous antioxidant system. However, due to many environmental, lifestyle, and pathological situations, excess radicals can accumulate, resulting in oxidative stress. Oxidative stress has been related to cardiovascular disease, cancer, and other chronic diseases that account for a major portion of deaths today. Antioxidants are compounds that hinder the oxidative processes and thereby delay or prevent oxidative stress. This article examines the process of oxidative stress and the pathways by which it relates to many chronic diseases. We also discuss the role that endogenous and exogenous antioxidants may play in controlling oxidation and review the evidence of their roles in preventing disease.

Keywords antioxidant, chronic disease, aging, oxidation, cardiovascular, vitamins

1. INTRODUCTION

The prevention of chronic disease has prompted much scientific research. Population studies have shown that up to 80% of cardiovascular disease, 90% of type II diabetes, and approximately 30% of cancers could be avoided by diet and lifestyle changes.[147] During the past few decades, scientific discovery has prompted debate as to whether oxidation, or more specifically, oxidative stress, is a primary cause or a secondary phenomenon of many chronic diseases as well as the aging process itself. Consequently, much scientific curiosity and resources have focused on the role that antioxidants play in hindering oxidation, thereby delaying or preventing oxidative stress. This article will examine the process of oxidative stress and the pathways through which it relates to many chronic diseases. We will also discuss the roles that endogenous and exogenous antioxidants may play in controlling oxidation and preventing disease.

Paper No. FSR 03-28 of the Journal Series of the Department of Food Science, North Carolina State University, Raleigh, NC 27695-7624.

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of products named, or criticism of similar ones not mentioned.

Address correspondence to George L. Catignani, Department of Food Science, North Carolina State University, Box 7624, Raleigh, NC 27695-7624, USA. E-mail: george_catignani@ncsu.edu

2. THE GENESIS OF OXIDATION AND THE PRODUCTION OF FREE RADICALS

Oxygen is a toxic mutagenic gas that appeared in significant amounts in the Earth's atmosphere over 2.5 billion years ago.[1] This rise in atmospheric oxygen led to the formation of the ozone layer that filtered out enough of the solar ultraviolet radiation to allow living organisms to leave the sea and inhabit the land. At the same time, the more primitive anaerobic organisms died out with the exception of those that evolved a defense mechanism to protect themselves from the toxicity of oxygen. Other surviving organisms continued to evolve by using oxygen for metabolic transformations and energy production in mitochondria. Multicellular organisms developed as systems evolved to distribute oxygen in a controlled manner. Most eukaryotic cells have an oxygen gradient, decreasing from the cell membrane to the mitochondria in which oxygen is consumed during aerobic

metabolism. Most human body cells are exposed to fairly low oxygen concentration, which may be regarded as an antioxidant defense mechanism.[2]

Mammals have developed mechanisms to insure that oxygen is transported to all the cells that need it. About 85–90% of the oxygen taken up from the plasma by animal cells is utilized by the mitochondria to produce adenosine triphosphate (ATP).[3] The essence of metabolic energy production involves the oxidation of food materials, i.e., the loss of electrons that are accepted by electron carriers such as nicotinamide adenine dinucleotide (NAD^+), flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD). These reduced compounds in turn are re-oxidized by oxygen in the mitochondria, producing ATP. The terminal enzyme in this electron transport chain, cytochrome oxidase, adds four electrons to oxygen. It is through a stepwise process that partially reduced oxygen species are generated (Figure 1).

A free radical is defined as any species capable of independent existence that contains one or more unpaired electrons. The presence of unpaired electrons makes free radicals highly reactive because they require another electron to fill the orbital and become stable.

When a single electron is added to the ground state O_2 molecule, the superoxide radical is produced (O_2^{-}). Several organic molecules oxidize in the presence of O_2 to produce the superoxide radical, including glyceraldehydes, the reduced flavins, adrenaline, L-dopa, dopamine, cysteine, etc. These autooxidations are catalyzed by the presence of metal ions such as iron and copper.

The most important source of superoxide radicals *in vivo* are the electron transport chains present in many bacterial membranes, within mitochondria, endoplasmic reticulum, and nuclear membranes in eukaryotic cells. Most of these radicals are produced by the incomplete transfer of electrons on O_2 prior to the terminal cytochrome oxidase step. This production of radicals is increased as the O_2 concentration increases. It is estimated that 1–3% of the O_2 reduced in mitochondria may form the superoxide radical.[4]

Superoxide production increases when the integrity of the mitochondrial electron transport chain organization is compromised. The production of these oxygen radicals may thus result in damage to the proteins, lipids, and DNA in the respiring mitochondria, leading to mutations in mitochondrial DNA that have been associated with a wide range of human diseases.[5]

In addition to the electron transport system release of electrons, the liver endoplasmic reticulum may generate superoxide radicals through the desaturase enzyme system. In this system, desaturase introduces C=C bonds into fatty acids. The reaction requires O_2, NADH or NADPH, and cytochrome b_5. Electrons are transferred from NAD(P)H to cytochrome b_5 by a flavoprotein enzyme. Then the reduced cytochrome b_5 donates electrons to the desaturase enzyme. Both cytochrome b_5 and the flavoprotein can leak electrons on to O_2, to produce the superoxide radical.

The production of superoxide radicals occurs within all aerobic cells and is dependent on oxygen concentration. The superoxide radical is relatively innocuous, but at physiological pH approximately 1% will be protonated to the more reactive peroxyl radical ($HO_2^{\cdot}$).[6] Superoxide can decrease the activity of certain enzymes including some antioxidant defense enzymes such as catalase, glutathione peroxidase, and several in the energy metabolism scheme such as NADH dehydrogenase (Figure 2).

Another enzymatic target of superoxide damage is the ribonucleotide reductase that makes the precursors required for DNA synthesis. Calcineurin, a protein involved in signal transduction, may also be damaged.

Aside from direct damage, superoxide can be more cytotoxic by generating other reactive species, such as hydrogen peroxide, H_2O_2, by the addition of one more electron. Hydrogen peroxide is not a radical since the additional electron fills the orbital, but it can attack certain enzymes such as glyceraldehyde-3-phosphate dehydrogenase, an enzyme in the glycolytic pathway (Figure 3). It can also oxidize certain keto-acids such as pyruvate. H_2O_2 leads to depletion of ATP, reduced glutathione, and NADPH. It induces a rise in free cytosolic Ca^{2+} and activates a polymerase that leads to cell death. Finally, H_2O_2 can cross cell membranes to react with iron and copper ions to form much more damaging species such as hydroxide radical ($OH^{\cdot}$) and peroxynitrite ($NO^{\cdot}$).[7]

Free radicals can also be generated by activated leukocytes as part of the immune response. Macrophages and neutrophils possess the enzyme NADPH oxidase which can catalyze the

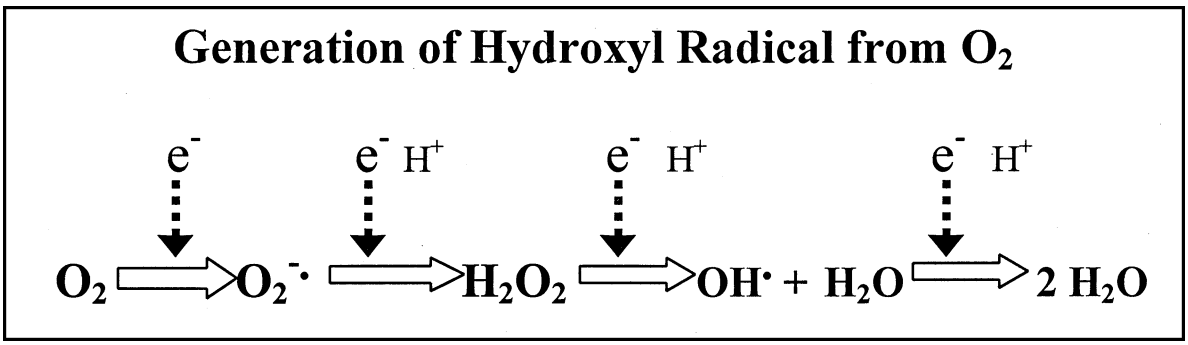

Figure 1 Step-wise generation of hydroxyl radical from oxygen. $O_2^{-\cdot}$ = superoxide radical, $OH^{\cdot}$ = hydroxyl radical.

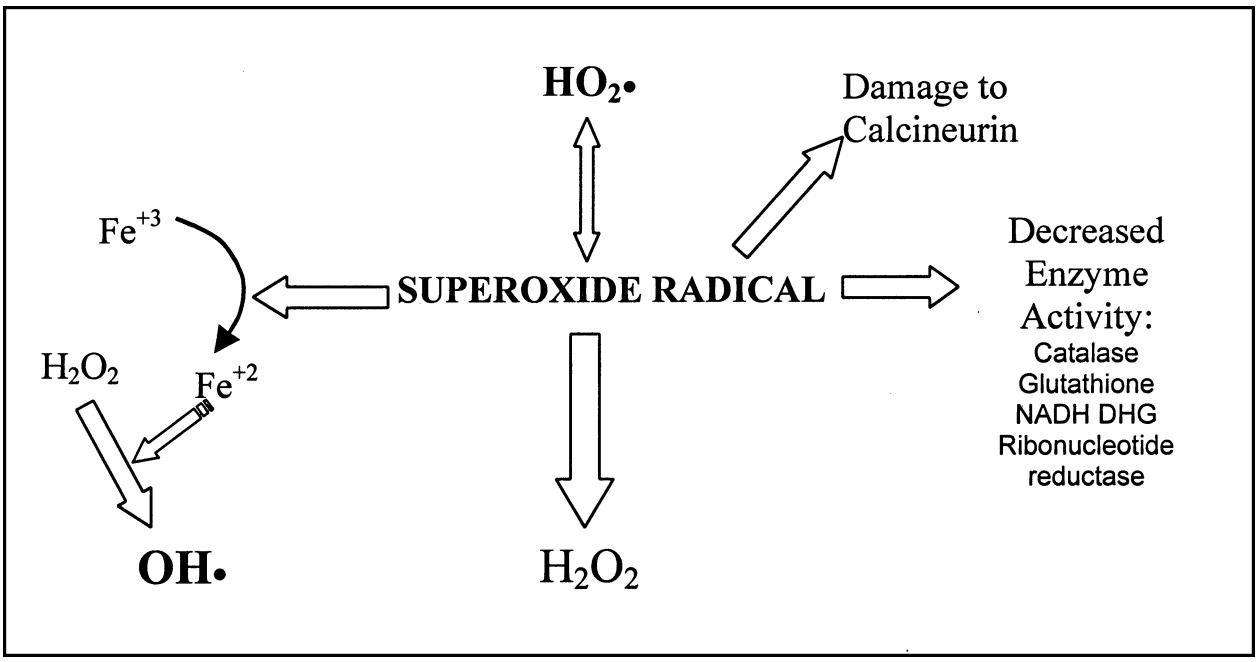

Figure 2 Target and fates of superoxide radical. DHG = dehydrogenase.

one-electron reduction of oxygen to superoxide which in turn generates the other oxygen radicals discussed. Another enzyme found in leukocytes, myeloperoxidase, generates hypochlorous acid (HOCl) from H_2O_2. These reactive oxygen species (ROS) are used in the immune response to kill ingested or extracellular bacteria. Unfortunately, their actions are not limited to their intended purpose and they may contribute to the detrimental effects seen by the free radical-induced oxidative process.[8]

Tissue damage will also uncouple electron transport chains and release compartmentalized reactions to generate free radicals. Ischemia, as occurs during myocardial infarction and organ transplants, can produce free radicals through the action of xanthine oxidase. When tissues are disrupted the normally present enzyme, xanthine dehydrogenase, can be converted into xanthine oxidase as –SH groups are oxidized or by limited proteolysis due to increased intracellular Ca^+ levels. Therefore, it

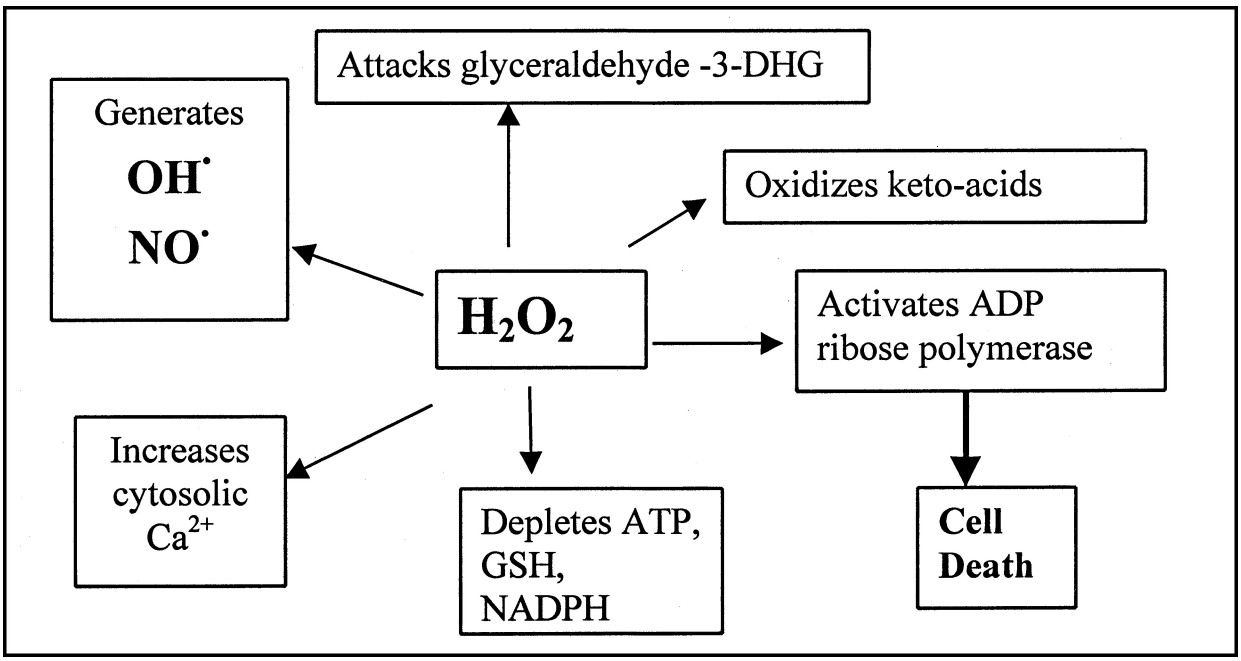

Figure 3 Targets and actions of peroxides *in vivo*. $OH^·$ = hydroxyl radical, $NO^·$ = nitric oxide radical.

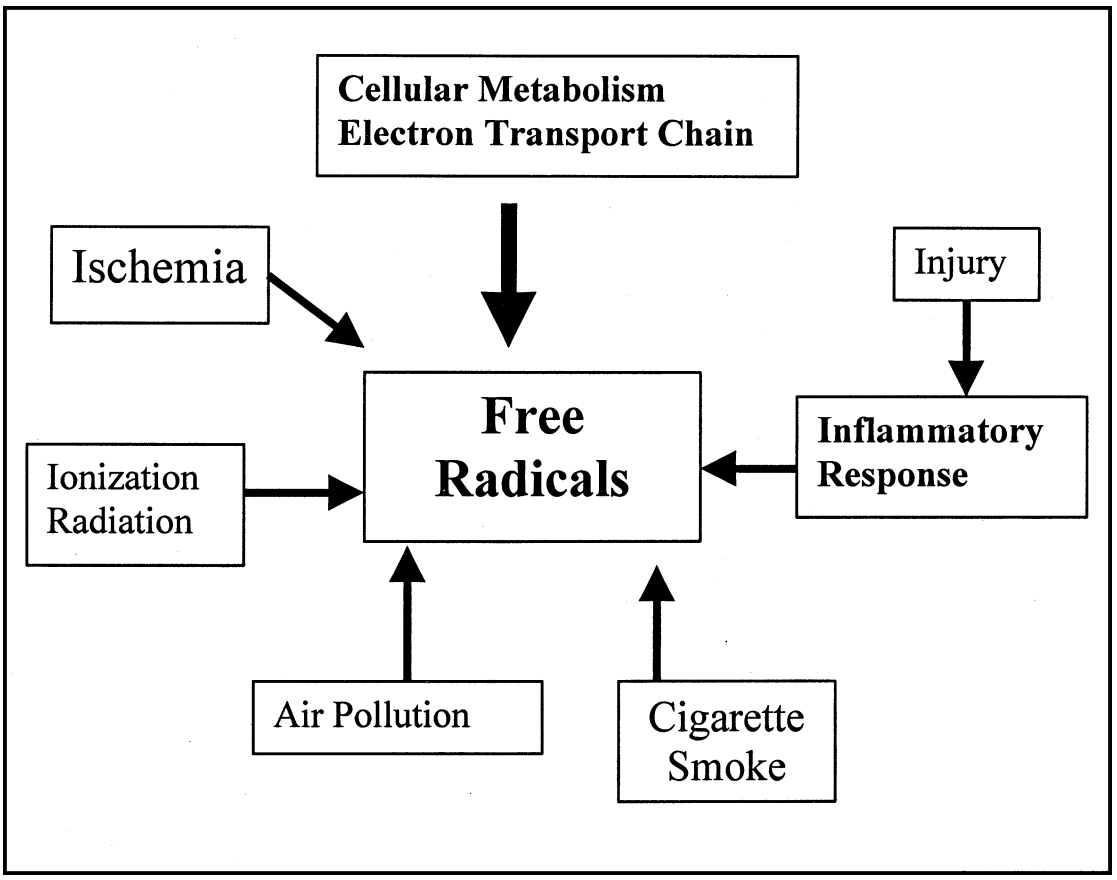

Figure 4 Summary of sources of free radicals.

is upon reperfusion of ischemic tissue with blood and oxygen that xanthine oxidase converts hypoxanthine and xanthine to uric acid thereby producing superoxide that may in turn produce peroxide and, with the input of reduced iron, make a hydroxyl radical. This plays a major role in the tissue damage seen in ischemia/reperfusion injury.[8] Other environmental factors also lead to the production of free radicals (Figure 4). Exposure to ultraviolet radiation can generate free radicals, as can air pollution and cigarette smoke. Nitrogen dioxide, one of the major oxidants in smog, is also found in cigarette smoke. Two free radicals are found in cigarette smoke, one in the tar portion and the other in the gaseous phase. The principal radical, $NO^{\cdot}$, found in the tar portion, is capable of reducing oxygen to the superoxide radical. The much more reactive oxygen and carbon-centered radicals are found in the gas phase.[9]

3. ANTIOXIDANT DEFENSES

An antioxidant has been defined by Halliwell and Gutteridge[2] as "any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate." When free radicals are generated *in vivo* many antioxidants act in defending the organism from oxidative damage (Figure 5).

As a first line of defense, the preventive antioxidants such as peroxidases and metal chelating proteins suppress the generation of free radials. Next, the radical-scavenging antioxidants such as vitamin C and vitamin E scavenge radicals to inhibit the oxidation chain initiation and prevent chain propagation as a second line of defense. This may also include the termination of a chain by the reaction of two radicals. The repair and de novo enzymes act as the third line of defense by repairing damage and reconstituting membranes. These include lipases, proteases, DNA repair enzymes, and transferases.[10]

3.1. Endogenous Antioxidants

There is a vast network of intracellular and extracellular antioxidants with diverse roles within each area of defense (Figure 6). Catalase converts H_2O_2 to O_2 and H_2O while superoxide dismutase (SOD) converts the superoxide radical to H_2O_2 and O_2. Some of the antioxidant enzymes exist in several forms. For example, membrane, cytosolic, and plasma forms of glutathione peroxidase have been isolated and SOD has membrane, cytosolic, and extracellular forms. The levels and locations of these antioxidants must be tightly regulated for cell survival. The

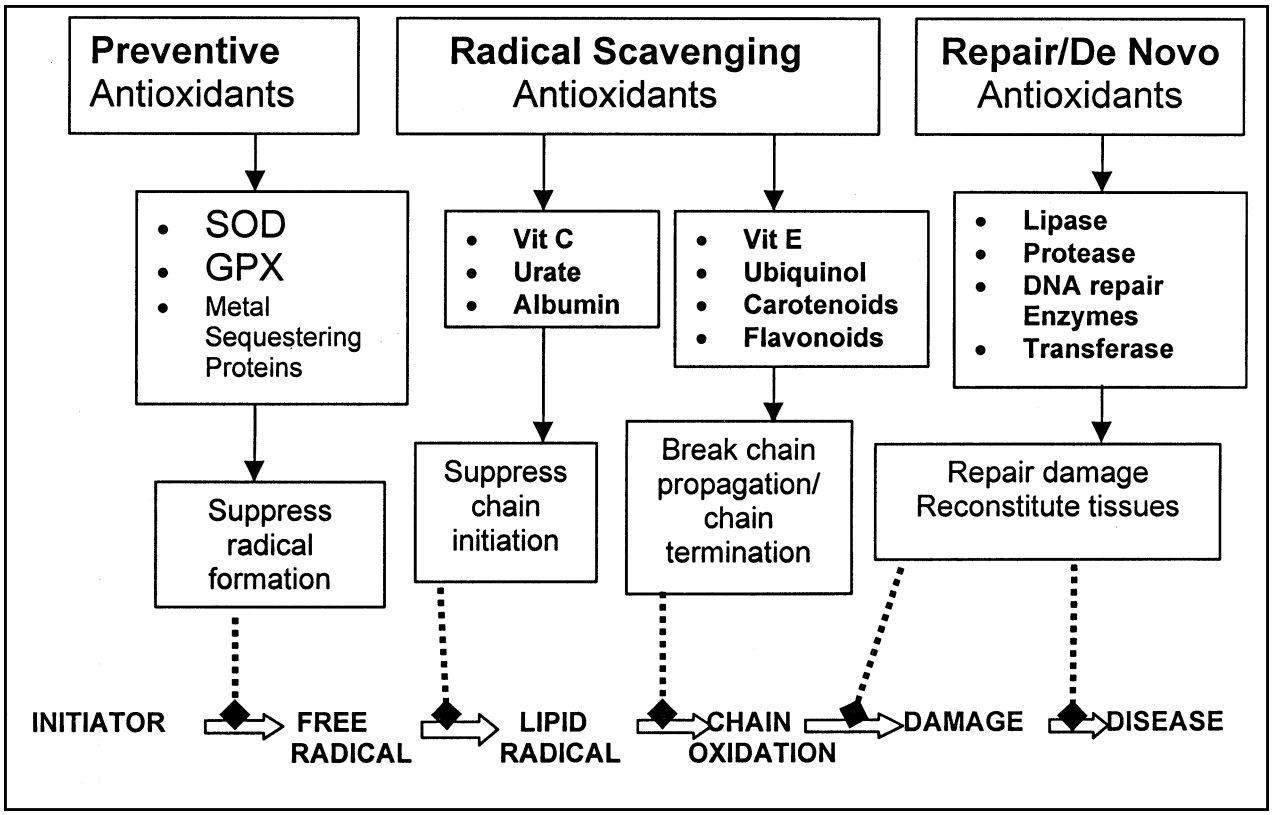

Figure 5 Antioxidant groups and actions. SOD = Superoxide Dismutase, GPX = Glutathione Peroxidase, dotted lines = suppression.

antioxidant enzymes, SOD, glutathione peroxidase (GPX), and catalase (CAT), work within the cells to remove most superoxides and peroxides before they react with metal ions to form more reactive free radicals. Peroxidative chain reactions initiated by free radicals that escaped the antioxidant defenses are terminated by chain-breaking water or lipid soluble antioxidants.[11]

3.2. Exogenous Antioxidants

Diet plays a vital role in the production of the antioxidant defense system by providing essential nutrient antioxidants such as vitamin E, C, and β-carotene, other antioxidant plant phenols including flavanoids, and essential minerals that form important antioxidant enzymes (Figure 7). For example, SOD contains zinc and glutathione peroxidase contains selenium.[8]

Diet also plays an important role in the oxidation process by affecting the substrates that are subject to oxidation. The best example is the oxidation of lipids. Polyunsaturated fatty acids (PUFA) having two or more double bonds are increasingly susceptible to free radical attack as the number of double bonds increases. Antioxidants available at the site of radical attack break the chain of oxidation by being preferentially oxidized by the attacking radical, thereby preventing oxidation of the adjacent fatty acid.

Membrane and lipoprotein fatty acid composition is determined primarily by diet. For example, consumption of meat yields more membrane arachidonic acid (20:4) while fish yields more eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6). On average, the vegetarian diet has less total fat and consequently, less total polyunsaturated fatty acids.[10] This could help to explain health benefits sometimes seen with the vegetarian diet. The beneficial effects of olive oil on LDL oxidation may be due to the predominance of monounsaturated fatty acids over PUFA and to the presence of antioxidants such as flavonoids and other phenolic compounds found in olives.[12]

4. DISEASES ASSOCIATED WITH OXIDATIVE STRESS

Under ideal circumstances the body would be in a steady state with free radicals produced and quenched by the endogenous antioxidants. However, it has been determined that this balance is not perfect because oxidative damage occurs to DNA, proteins, lipids, and small molecules in living systems under ambient oxygen states. Oxidative stress refers to the situation in which there is a significant imbalance between free radicals and the antioxidant defense system. The resulting harm is termed oxidative damage.

Cells can normally deal with mild oxidative stress by up-regulating the synthesis of antioxidant defense mechanisms through changes in gene expression. However, at higher levels of oxidative stress, cell injury may occur when adaptation is not adequate for the build up of oxidation products. This leads to oxidative damage to all types of biomolecules including DNA,

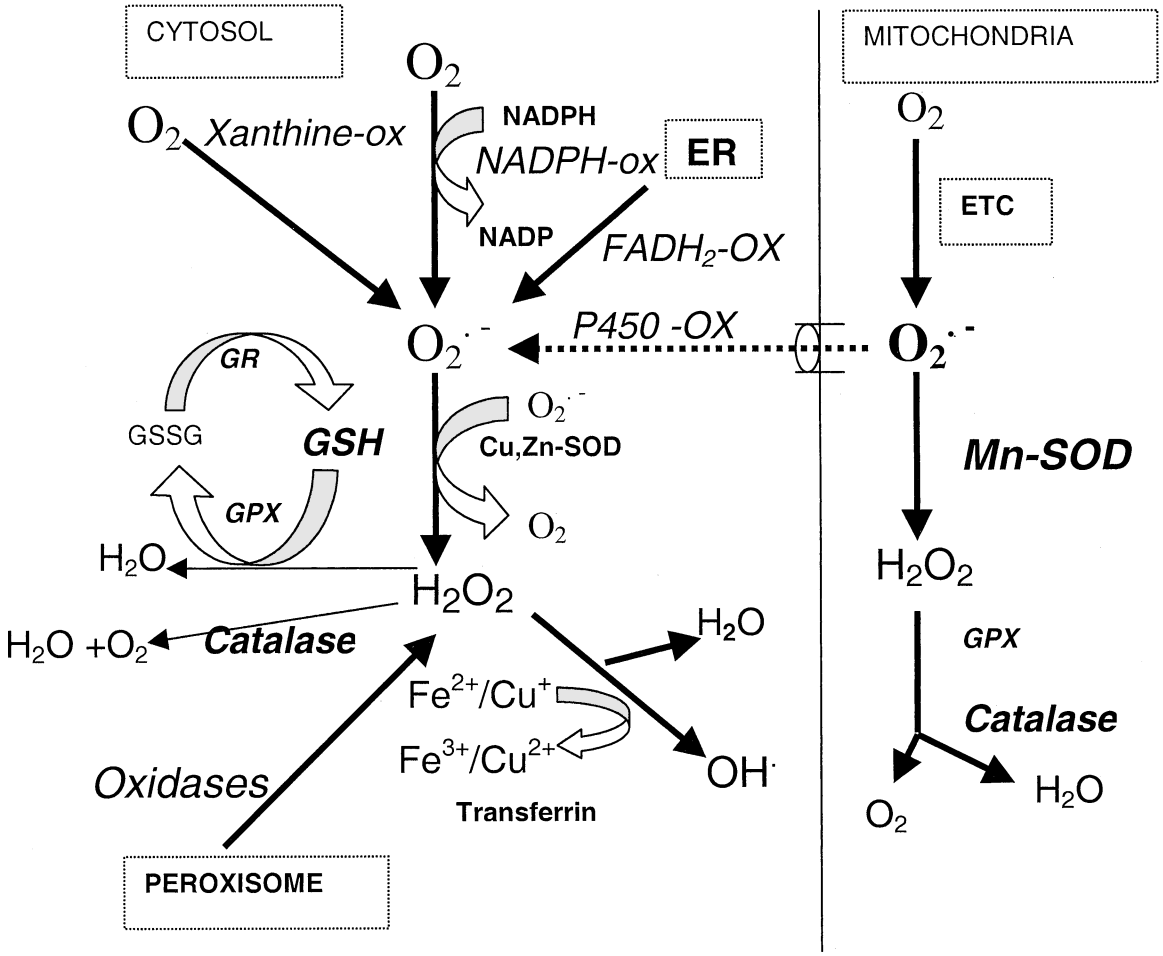

Figure 6 Generation of reactive oxygen species and endogenous antioxidant mechanisms. ER = endoplasmic reticulum, ETC = electron transport chains, OX = oxidases, endogenous antioxidants are bolded: GSH= reduced glutathione, GSSG = oxidized glutathione, GR = glutathione reductase, GPX = glutathione peroxidase, SOD = superoxide Dismutase.

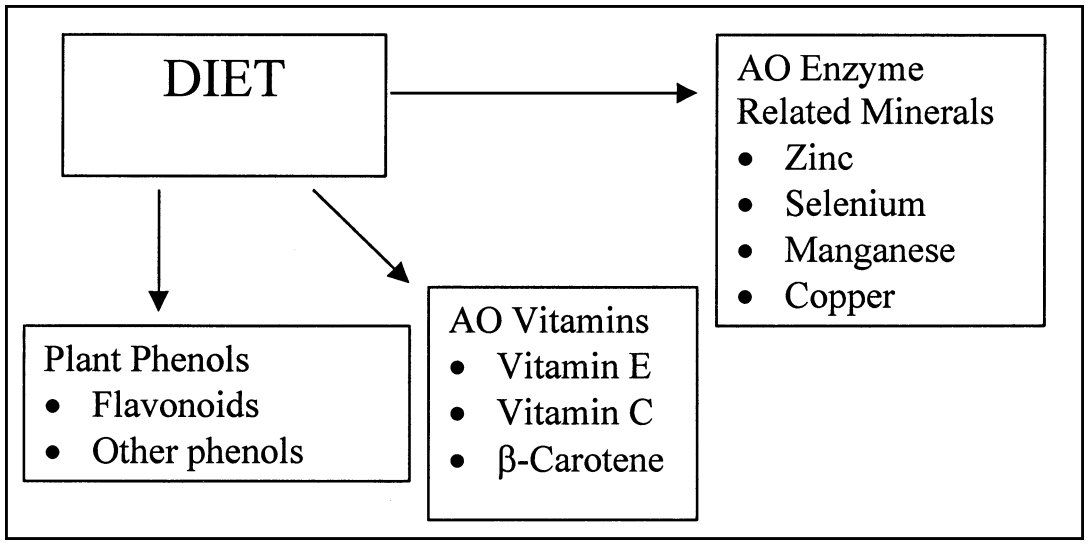

Figure 7 Contribution of diet to antioxidant defenses. AO = Antioxidant.

proteins, and lipids that have been associated with many diseases. The target of oxidative damage varies depending on the characteristics of the cell and the type and degree of stress imposed. Whereas some diseases may be caused by oxidative damage to proteins, DNA, and lipids, oxidative stress may be a consequence and not a cause of the primary process in many others. As a secondary event, however, oxidative stress plays an important role in furthering tissue damage in several diseases. Tissue damage by infection, trauma, toxins, temperature extremes, and other causes usually leads to the formation of increased amounts of free radicals that contribute to disease pathology.[13] The imbalance of reduction-oxidation homeostasis appears to be one of the processes that regulates gene expression in many pathological conditions.[14]

4.1. Cardiovascular Disease

Cardiovascular disease is the leading cause of death in the United States, Europe, and Japan.[15] Most cardiovascular events are secondary to atherosclerosis, a disease of the arteries involving a local thickening of the vessel wall, mainly evident in mid-sized muscular arteries.[16] Three types of pathological entities are generally recognized: foam cells, fatty streaks, and fibrous plaques. A stroke or myocardial infarction occurs when the lumen of the vessel becomes completely occluded, usually by a thrombus forming at the site of a plaque.

Atherosclerotic lesions are thought to be initiated by emigration of monocytes into the arterial inner core (tunica intima), recruited by adhesion molecules, possibly in response to arterial endothelium injury.[17] A variety of factors have been implicated in causing this initial injury, including mechanical damage from flow stress worsened by high blood pressure, viral infection (herpes viruses and cytomegalovirus), exposure to blood-borne toxins such as xenobiotics from cigarette smoke, and elevated levels of normal metabolites, such as glucose, cholesterol, or homocysteine.[13]

The fatty streak is the earliest and most common atherosclerotic lesion seen in cardiovascular disease. It is made up of aggregates of foam cells in the subendothelial portion of the vessel wall. These foam cells contain large deposits of oxidized LDL cholesterol engulfed by macrophages, which were formerly monocytes circulating in plasma before diffusing through the intimal wall of the blood vessel. *In vitro* work has shown that the receptors of these scavenger macrophages identify oxidized LDL and engulf it in an unregulated manner, thereby transforming the macrophage into a lipid-laden foam cell (Figure 8). These transformed macrophages are considered as precursors to the development of the occlusive plaque of atherosclerosis.[18]

The following mechanisms have been observed: (1) increased recruitment of circulating monocytes into the vessel intima by the chemoattractant force of oxidized LDL,[19] (2) enhanced rates of oxidized LDL uptake and degradation by the macrophage through the receptor,[20] (3) the oxidation of the LDL particle inhibits macrophage exodus from the artery,[21] and (4) cellular injury caused by oxidized LDL.[22] These observations have led to the hypothesis, not yet proven, that *in vivo* LDL may be oxidized by free radicals, leading to the atherosclerotic process. However, LDL from human atherosclerotic lesions has been shown to contain lipid peroxidation products not found in normal vessels.[23] Elevated amounts of oxidized LDL are present in the

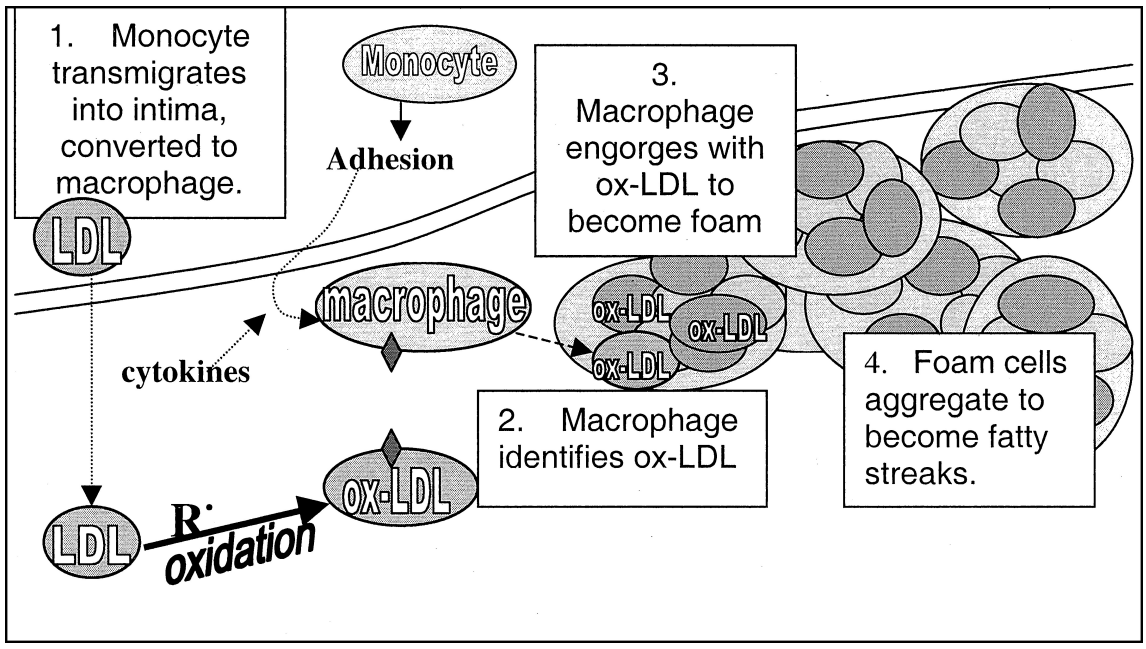

Figure 8 Proposed scheme of fatty streak development through LDL oxidation. Adapted from Willcox et al. 2003.[133] R· = Free Radical, ox-LDL = Oxidized LDL cholesterol.

blood of patients with atherosclerotic disease,[24] and antibodies against oxidized LDL react with rabbit and human atherosclerotic lesions.[25] The presence of autoantibodies against oxidized LDL has been correlated with atherosclerosis.[26,27] In contrast, native or non-oxidized LDL uptake is under high homeostatic regulation. Uptake is down regulated by an excess of intracellular cholesterol.

A. The Initiation of Lipid Peroxidation

Dietary fats, after being digested and absorbed, are transported through the body via lipoproteins. The more cholesterol rich lipoprotein, LDL, contains approximately 2700 fatty acids/molecule, about half of which are PUFAs, that are very sensitive to oxidation.[28] Polyunsaturated fatty acids are also found in cell membranes where their side chains mainly determine cell membrane fluidity. Membrane fluidity is essential for the proper function of biological membranes, including the action of many important receptors. The fluidity of cell membranes decreases with lipid peroxidation.[29]

Increased concentrations of superoxide, hydrogen peroxide, and the presence of metal ions can create conditions favorable for the production of hydroxyl radical ($OH^{\cdot}$) which results from the addition of one more electron to H_2O_2.

The most reactive species, hydroxyl radical ($k = 10^9\ M^{-1}\ s^{-1}$), is capable of interacting with almost every type of molecule found in living cells.[30] It reacts by hydrogen abstraction, addition, and electron transfer from non-radical molecules, thus initiating the oxidation of macromolecules such as lipids resulting in a lipid radical ($L^{\cdot}$) that in the presence of oxygen generates a lipid peroxyl radical ($LOO^{\cdot}$). This $LOO^{\cdot}$ can continue the chain of oxidation by attacking another lipid and the oxidation process continues. Lipid peroxidation begins when a methylene group (—CH2—] of the PUFA is attacked by a free radical to abstract a hydrogen atom and an electron. An adjacent double bond weakens the attachment of the hydrogen atoms present on the next carbon, especially if there is a double bond on either side of the methylene group. With subsequent rearrangement and reaction with more oxygen, lipid peroxyl radicals ($LOO^{\cdot}$) are formed. The lipid peroxyl radicals can oxidize adjacent lipids in the cell membrane or LDL molecule. Now oxidatively modified, the LDL cholesterol particle is identified by the receptor of the macrophage and engulfed, leading to foam cell formation as discussed above.[16] Through this chain reaction, a single initiating radical can result in the conversion of hundreds of fatty acid side chains into lipid peroxides that alter the integrity and biochemical function of cell membranes (Figure 9).[31]

Another fate of the oxidized fatty acid is the formation of a cyclic peroxide that continues to be oxidized to malondialdehyde, F_2-isoprostanes, or other oxidation products.[32]

B. Epidemiological Studies

The WHO/MONICA Study, reported by Gey et al. in 1991,[33] provided some of the first evidence for the hypothesis that antioxidants could reduce the risk for cardiovascular disease. In this cross-cultural study of 16 countries, both the blood levels of α-tocopherol and the tocopherol/cholesterol ratio inversely correlated with mortality rates. However, vitamin C and β-carotene were found not to be protective.

The Health Professionals Study assessed the intake of vitamin C, β-carotene, and vitamin E in 39,910 male health professionals.[34] During 4 years follow up, 667 cases of coronary disease were observed. After controlling for several risk factors, there was a 39% reduction in major coronary events in the highest intake category for vitamin E as compared to the lowest. Men who took at least 100 IU of vitamin E per day for at least 2 years had a relative risk of 0.47–0.84 compared to those who did not take a supplement. Of the 4,814 men who reported previous history of cardiovascular disease (CVD), there was a modest, but non-significant inverse association between intake of flavonoids and subsequent coronary mortality rates.[35] β-Carotene intake was not associated with a lower risk among non-smokers, but it was associated with lower risk in smokers. A high intake of vitamin C was not associated with a lower risk of coronary disease.

The Nurses Health Study involved 87,245 women aged 34–59, free of obvious cardiovascular disease at the time of entry.[36] Again, detailed dietary questionnaires were used to assess intake of antioxidant nutrients, including vitamin E. During the 8 year follow-up period, 437 non-fatal heart attacks occurred as well as 115 deaths due to coronary disease. The participants in the top quintile of dietary vitamin E intake showed 23–50% less heart disease after correction for age and smoking. Participants who took supplements of vitamin E for at least 2 years had a relative risk of major CVD events of 0.38–0.91 (mean: 0.59) after adjustment for age, smoking, other risk factors, and intake of other antioxidant nutrients. There was no correlation between reduced risk and intake of vitamin C or β-carotene.

A National Institute of Aging study, Established Populations for Epidemiological Studies of the Elderly (EPESE), involved four communities in the eastern part of the United States. Approximately 11,000 subjects between the ages of 67 and 105 were interviewed twice, 3 years apart, and then followed for an average of 6 years. There were 3,490 deaths during the follow-up period. The use of vitamin E supplements was associated with a significantly reduced relative risk for all causes of mortality of 0.66 and risk of death from heart disease was 0.53. Persons reporting the simultaneous use of vitamin E and vitamin C supplements had a relative risk all-cause mortality of 0.58 and for coronary heart disease mortality of 0.47, but there was no significant interaction between the two antioxidant vitamins.[37]

The Iowa Women's Health Study followed 35,000 postmenopausal women for an average of 7 years.[38] A 62% reduction in CVD deaths was found in those in the highest quintile of vitamin E intake from food. No significant association was seen when the intake of vitamin E from supplements were evaluated. However, no information was collected on the length of time

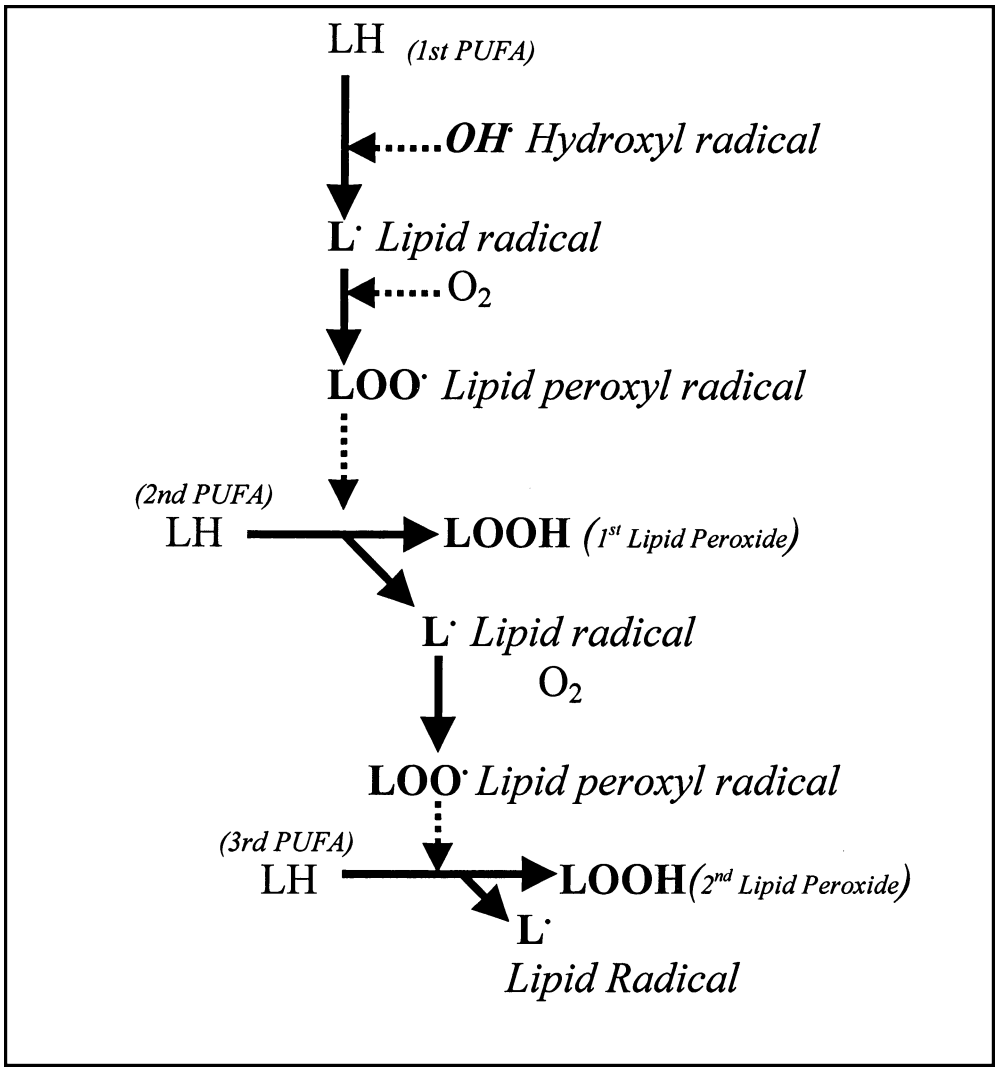

Figure 9 The liped peroxidation chain reaction.

supplements were used. There was no reduced risk of cardiovascular disease seen with vitamin C or β-carotene intake.

In a Finnish prospective study, 2,748 men and 2,385 women were followed for an average of 14 years.[39] A comparison was made between risk of heart disease among those in the highest tertile of vitamin E intake versus those in the lowest. Men in the highest category had a 34% (non-significant) reduction of risk of coronary heart disease (CHD) death while women had a 65% (significant) reduction. A reduction in risk was also seen with intake of β-carotene.

The Zutphen Elderly Study was a longitudinal investigation of risk factors for chronic disease using a cohort of 805 men aged 65–84 living in the eastern Netherlands.[40] A dietary history was conducted and the subjects were followed for 5 years. Flavonoid intake was significantly inversely associated with mortality from cardiovascular disease and showed an inverse but non-significant relation with incidence of myocardial infarction. The relative risk of CVD mortality in the highest versus lowest tertile of flavonoid intake was 0.42. After adjustment for intake of other antioxidants, and significant variables, the risk was still significant.

Flavonoid intake was also inversely associated with mortality from CVD in the Seven Countries Study.[41] and in a Finnish study, conducted between 1972 and 1992.[42]

A 1990 study conducted in France found that people who drink wine daily had a lower incidence of heart disease than their counterparts in the U.S. and other Western countries despite a high fat diet.[43] This observation, termed the "French Paradox," prompted further study which indicated that aside from the cardioprotective effect of ethanol, antioxidant plant phenols may explain the lower incidence due to reduced LDL oxidation.[44]

Finally, an analysis of the Nurses' Healthy Study and the Health Professionals' Follow-up Study revealed a 4% decrease in relative risk for coronary disease with every daily serving of leafy green vegetables and vitamin C rich fruits.[45]

C. Clinical Trials

Several clinical studies indicate that supplementation with antioxidants such as Probucol, vitamin C, and vitamin E, but not β-carotene, increases the resistance of LDL to oxidation *ex vivo*. However, it is not clear whether they also reduce the severity of atherosclerosis. A few randomized, double blind, placebo-controlled human trials have given inconsistent results.

The Cambridge Heart Antioxidant Study (CHAOS) trial in England evaluated the effect of vitamin E supplementation (400 or 800 IU/day) or placebo on the risk of myocardial infarction in 2,000 male and female patients with evidence of coronary atherosclerosis.[46] Results demonstrated that vitamin E supplementation significantly decreased the risk of nonfatal myocardial infarction (MI) and cardiovascular related mortality by 47% after 200 days.

A randomized intervention trial involving almost 30,000 adults from Linxian, China evaluated the effects of supplementing with specific combinations of antioxidant vitamins over a 5 year period.[47] Beta-carotene, selenium, and vitamin E taken together had a marginally significant effect in reducing total mortality and a trend towards a reduction of CVD mortality (−9%). No effect was seen with vitamin C.

Another randomized double-blind study investigated the effects of antioxidant supplements on the incidence of lung cancer in Finland. The Alpha Tocopherol Beta Carotene (ATBC) trial enrolled 29,133 male smokers between the ages of 50 and 69 that had smoked an average of 36 years.[48] The study participants received daily supplements of 20 mg synthetic β-carotene, 50 mg synthetic vitamin E, both, or a placebo and were followed for 5 to 8 years. Analysis of heart disease in the ATBC Study involved 27,271 participants with no prior history of MI. After 6 years, coronary events were reduced by 4% in those supplemented with vitamin E, however they were increased by 1% in those receiving β-carotene. Vitamin E decreased CVD deaths by 8% while β-carotene had no effect.

A similar study, the Carotene and Retinol Efficacy Trial (CARET), examined the effects of antioxidant supplementation on the risk of lung cancer in smokers, ex-smokers, and workers exposed to asbestos. Participants were given 30 mg β-Carotene and 25,000 IU vitamin A or a placebo over 4 years.[49] Once again, the relative risk of death from CVD increased, though non-significantly, in the supplemented group as compared to placebo. However, the trial had to be terminated early because of an increased incidence of lung cancer in the treatment group.

The Gruppo Italiano per lo Studio Della Sopravvivenza nell'Infarto Miocardico Study (GISSI) is a recently completed secondary prevention trial that involved 11,324 patients who had survived an MI within the 3 month period prior to study enrollment.[50] There were 4 treatment groups: 300 mg/d of synthetic vitamin E, 0.9 g/d of a mixture of n-3 PUFA consisting of a 2:1 ratio of docosahexaenoic acid, and eicosapentanoic acid esters, or both, or neither. The n-3 PUFA supplemented group experienced a significant reduction in overall mortality, nonfatal MI, and stroke over the 3.5 year follow-up period which was not further improved with the addition of vitamin E. However, vitamin E supplementation alone resulted in a 20% reduction in cardiovascular deaths and a 35% reduction in sudden death.

Another study reported in the New England Journal of Medicine,[51] enrolled 2,545 women and 6,996 men 55 years of age or older who were at high risk for CVD. These patients were randomly assigned to treatment groups receiving either 400 IU (natural) vitamin E or placebo, and either an angiotensin-converting enzyme inhibitor (Ramipril), or placebo. Over an average of 4.5 years there were no significant differences in deaths from cardiovascular disease, unstable angina, congestive heart failure, or other secondary outcomes for those assigned the vitamin E supplement compared to the placebo.

The SECURE (Study to Evaluate Carotid Ultrasound Changes in Patients Treated with Ramipril and Vitamin E) Study was a prospective double blind study of the HOPE (Heart Outcomes Prevention Evaluation) trial. This study evaluated the effects of long term treatment with the angiotensin-converting enzyme inhibitor, Ramipril and vitamin E on atherosclerosis progression in 732 high risk patients over a 4.5 year period. There were no significant changes in the progression of atherosclerosis between patients treated with 400 mg vitamin E and those receiving a placebo.

The Vitamin E Atherosclerosis Prevention Study (VEAPS) found that 400 IU/day of supplemented vitamin E increased plasma levels of the vitamin and reduced oxidation parameters, but did not reduce intima-media thickness.[52]

Recently reported was a randomized placebo-controlled study of 20,536 high risk individuals supplemented with 600 mg vitamin E, 250 mg vitamin C, and 20 mg β-carotene daily. Although plasma levels of each supplemented vitamin increased substantially, there were no significant reductions in the 5 year incidence of or mortality from any type of vascular disease, cancer, or other major outcome.[53]

A systematic review of studies involving antioxidant vitamins in the prevention of cardiovascular disease was reported in 2002. This review included human case control, cohort, and randomized controlled studies reported between 1989 and 2001 involving carotene, ascorbic acid, and vitamin E. Odds ratios of cohort studies (high versus low intake) were: β-Carotene 0.88 (n = 38,768), ascorbic acid 0.89 (n = 50,000), and α-tocopherol 0.77 (n = 82,379). In cohort studies of high versus low serum/plasma levels odds ratios were: β-carotene 0.46 (n = 5,061), ascorbic acid 0.56 (n = 13,018), and α-tocopherol 1.61 (n = 286). The randomized clinical trials (RCT) reported odds ratios of: β-Carotene 1.02 (n = 86,056), ascorbic acid 0.98 (n = 16,700), and α-tocopherol 0.96 (n = 48,346).

In observational studies, people with higher intake of the antioxidant vitamins usually had a lower risk of MI and stroke. In the RCT these antioxidant vitamins showed no risk reduction of these disease entities.[134]

4.2. Cancer

Early interest in the relationship between diet and cancer risk focused on dietary sources of the carcinogens themselves. However, results from epidemiological studies conducted in the 1960's and 1970's shifted interest to the idea that diet may provide some potential protection from cancer.[54] There is now evidence that micronutrients such as the antioxidants β-carotene, vitamin E, vitamin C, and selenium are associated with reduced cancer risks.[55] For example, out of 21 lung cancer studies, 15 found a significant inverse association with β-carotene while 4 found inconsistent results and 4 found no association. Highly invasive or metastatic cancer cells may require a certain level of oxidative stress to maintain a balance between proliferation and apoptosis. These cells generate large amounts of hydrogen peroxide that function as signal molecules that are involved in the survival of cancer cells. Antioxidants may suppress these hydrogen peroxide signal molecules and thereby inhibit cancer cell proliferation.[56]

It is estimated that 88–90% of human cancers are environmentally induced, approximately 35% by diet.[57] Much experimental data indicate that free radicals have a role in the initiation and promotion of cancer,[58] which involves changes in DNA either as a result of an inherited genetic anomaly or damage to the DNA strand. In view of the association between DNA damage and carcinogenesis, it is likely that any agent capable of modifying DNA could be carcinogenic. Free radicals fall into this category.

Hydroxyl radical ($OH^{\cdot}$) attack on DNA generates a series of modified purine and pyrimidine bases. It is estimated that oxidative lesions in DNA in normal cells average 1 per 10^6 bases, a value that is even higher than the levels of adducts of known carcinogens that are detected in carcinogen-exposed cells. This implies that endogenous damage to DNA by free radicals is an important contributor to the age-related development of cancer.[59,60]

An important factor in cancer development may be related to mutations in the p^{53} tumor-suppressing gene, a transcription factor that acts to block cell division. If p^{53} genes are inactivated, then cells can enter the cell cycle with damaged DNA. Mutations of p^{53} are found in 50% of cancer lesions. For example, about 75% of colorectal cancers and 90% of squamous cell skin cancers have mutations on the p^{53} gene. Oxidative damage may account for some of the C to T and G to A changes often seen in the p^{53} gene in human cancer.[61]

The most prevalent product of oxidative DNA damage is 8-hydroxy-2′-deoxyguanosine (8-OHdG). It is considered to be a reliable biological marker for oxidative stress.[62] The role of oxidation in DNA damage has been established by several *ex vivo* and *in vivo* animal and human studies in which 8-OHdG, 5-hydroxymethyluracil, and other DNA oxidation products were measured.[63–65]

Other than direct damage to DNA by free radicals, oxidative damage to lipids and to proteins such as DNA repair enzymes could also lead to DNA mutations as outlined in Figure 10.

A. Epidemiological Studies

Epidemiological data show a very strong inverse relationship between consumption of fruits and vegetables and the risk of cancer with an overall risk reduction between 30 and 50%.[66] Of 156 retrospective and prospective studies, 128 found significant protective effects of fruit and vegetable intake. In most of the cancer sites studied, and after controlling for confounding factors, subjects with the lowest consumption of fruits and vegetables experienced approximately twice the risk for cancer as compared to those with high intake. This protection was assumed to be related to β-carotene intake until intervention trials revealed conflicting findings.[67]

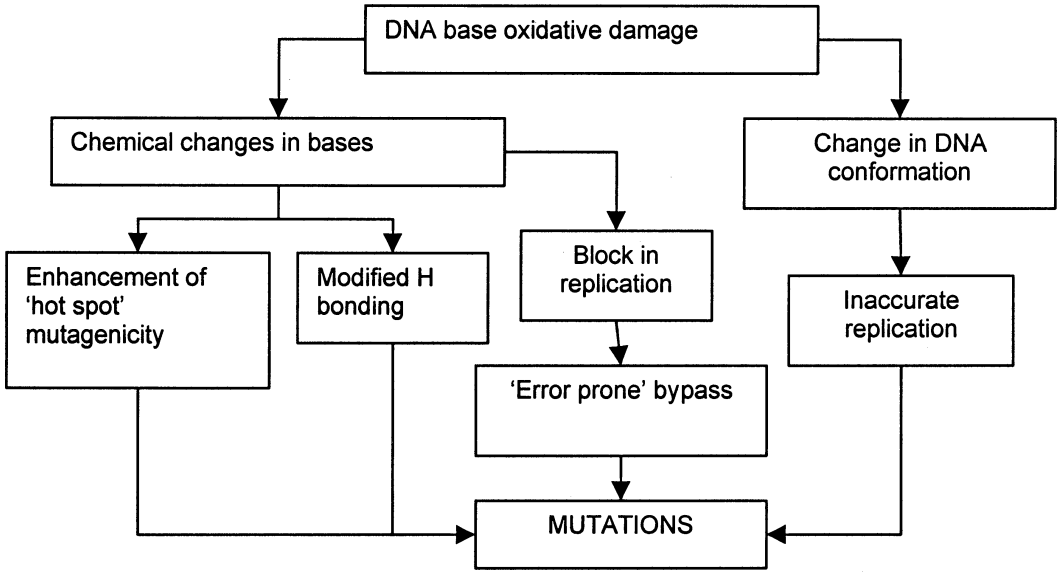

Figure 10 How structural changes in DNA can cause mutations. Adapted from Halliwell and Gutteridge, 1999.[2]

Table 1 Major components of antioxidant defense system. Adapted from Halliwell and Gutteridge, 1999.[2]

	Antioxidant action
Enzymes	
Superoxide Dismutase (SOD)	Removal of superoxide radical
Catalase	Reduction of H_2O_2 to water
Glutathione Peroxidase	Reduction of H_2O_2 to water
Thioredoxin	Reduction of peroxides
Metal ion sequestration	
Metallothionein	Chelates Zn, Ag, Cu, Cd, Hg
Phytochelatins	Chelates Cd, Zn, Cu
Transferrin	Chelates Fe
Albumin	Chelates Fe and Cu
Low molecular mass (endogenous)	
Urate	Scavaneges $NO_2^{\cdot}$
Note: the following have been shown to be antioxidants *in vitro*, but uncertain *in vivo*: Bilirubin, α-keto acids, sex hormones melatonin, coenzyme Q, lipoic acid, carnosine, anserine, melanins	
Low molecular mass (exogenous)	
Ascorbic Acid	Spares tocopherol, scavenges free radicals.
Vitamin E	Scavenges peroxyl radicals, most important chain breaking inhibitor of lipid peroxidation.
Carotenoids	*In vivo* antioxidant role uncertain
Plant phenols	Suggested but not proven to inhibit LDL oxidation *in vivo*.

Fruits and vegetables contain numerous substances with antioxidant activity including carotenoids, vitamin C, and polyphenols and many studies have examined the association of these nutrients with various cancers. For example, lung cancer is the most prevalent cancer worldwide and in the United States. Several have studied an association between lung cancer and intake and blood levels of β-carotene, vitamin C, vitamin E, and selenium. Individual studies suggest protective associations for each, but the totality of the evidence of an association at present is not convincing for any one of these antioxidant micronutrients.[135]

Several studies link vitamin C intake with reduced risk of several cancers, including oral, esophageal, stomach, colon, and lung.[66] Nine of eleven investigators studying the role of vitamin C in lung disease found a significant reduction with high intake even after controlling for smoking. Eight studies reporting on vitamin C intake and cancers of the esophagus and oral cavity found significant reduced risk with higher intake of the vitamin. Similar results were seen in studies regarding stomach cancer. A meta-analysis[68] of 12 major breast cancer studies found a strong and significant protective association with vitamin C intake.

Other studies have linked the intake of β-carotene with reduced risk of several cancers, especially lung and stomach.[69] A strong inverse relationship has been shown between lycopene with reduced risk of several cancers, especially prostate, lung, and stomach.[70] However, some data show that β-carotene may act as a co-carcinogen, especially in individuals exposed to cigarette smoke and other carcinogens found in industrial settings.[71] Several epidemiological studies also support the hypothesis that selenium status is inversely related to the risk of some kinds of cancer.[72]

Two studies have demonstrated that free radical-induced DNA damage is greater in cancerous compared to non-cancerous prostate tissue. Some dietary nutrients with antioxidant properties may prevent or slow progression of prostate cancer.[73]

B. Clinical Trials

The individual and collective effects of β-carotene (25 mg daily), vitamin C (1 gram daily), and vitamin E (400 mg daily) were studied in a randomized controlled clinical trial of 864 adults.[74] This trial lasted 4 years and used a primary end point of colorectal adenoma, a precursor of invasive cancer. There was no evidence that any of these vitamins reduced the incidence of colon cancer. However, a later analysis of the data revealed that study subjects who neither smoked cigarettes nor drank alcohol had a marked decrease in the risk of recurrent adenomas (RR = 0.56) with β-carotene supplementation while smokers (RR = 1.36) and drinkers (RR = 1.13) were at increased risk.[75] These findings suggested that β-carotene may act as a co-carcinogen in this population of smokers and drinkers. In an animal model, supplementation with β-carotene significantly increased carcinogen activating enzymes in the lung. It has been proposed that while functioning as an antioxidant, β-carotene may act as an anticarcinogen, but its oxidized products that are higher in oxidatively stressed situations such as smoking, can actually promote carcinogenesis.[71] In another study of humans[76] subjects were fed a high fruit/vegetable diet (12.5 servings/day) for 14 days. Serum levels of α- and β-carotenoids as well as lutein increased significantly. Tocopherol levels did not increase. Urinary and lymphocyte 8-OHdG decreased significantly with intervention. Urinary 8-epiprostaglandin F 2 (8-EPG) (a prostanoid epimer and marker of lipid peroxidation) decreased by 33%. Change in urinary 8-EPG was significantly correlated to change in 8-OHdG, supporting the concept that lipid peroxidation and DNA damage are related and that overall oxidative stress is reduced by high fruit/vegetable intake. The results of this small study, although significant, should be regarded as preliminary. What remains to be determined in a clinical trial is whether there is a direct correlation between incidence of cancer, DNA oxidation, and serum levels of antioxidant nutrients.

Evidence is growing that antioxidants may be beneficial as an adjunct to certain types of chemotherapy. A randomized clinical trial is currently underway to study this in cases of newly diagnosed ovarian cancer.[77]

4.3. Diabetes

Data from experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of both

Type 1 and Type 2 diabetes.[78] People with diabetes have an increased risk of atherosclerosis that is partially explained by an increased oxidizability of LDL.[79,80] This increased oxidative stress is accompanied by a decreased antioxidant capacity. There is disagreement as to whether serum α-tocopherol is lower, but there is general agreement that vitamin C levels are below normal in diabetic patients. Diabetic patients tend to have higher plasma lipid peroxides and higher markers of oxidation, such as F_2-isoprostanes.[81]

Diabetic complications may also be explained by increases in DNA damage due to oxidative stress. DNA damage was measured in 10 normal and 10 diabetic patients. A significant elevation in DNA strand breaks and oxidized pyrimidines was seen in patients with Type I diabetes compared with normal subjects. Altered purines showed a strong positive correlation with blood glucose level.[136]

Glycation of proteins leading to the accumulation of advanced glycation end products (AGEs) is known to be one of the sources of free radicals and has been strongly linked to the presence of diabetic complications[82] as seen in Figure 11. These glycation products may directly release superoxide radical and H_2O_2, activate phagocytes, and reduce glutathione levels.[83]

A. Clinical Trials

In a dietary intervention study, 10 stable Type II diabetic patients were placed on a flavonol (quercetin) rich diet for two weeks, after following a low flavonol diet for the preceding two weeks. Lymphocytes were subjected to an oxidative challenge *ex vivo* and DNA damage was measured. DNA damage was significantly reduced following consumption of the high flavonol diet, compared to the low flavonol diet.[84]

Studies in humans have demonstrated beneficial effects of various antioxidants in one of the complications, diabetic neuropathy. One such study supplemented Type I and II diabetics with α-lipoic acid, vitamin E, or selenium for 12 weeks. Results showed improvement in neurological symptoms and decreased lipid peroxidation.[135]

A randomized, double-blind, placebo-controlled clinical trial supplemented 28 Type I diabetic patients with a flavonoid based antioxidant medication while following a standardized 1,800–2,000 calorie diet.[85] Baseline measurements were made at the beginning of the trial, and reassessed after 3 months. Glycated hemoglobin (HbA_{1c}) values decreased slightly but significantly whereas the decline was non-significant in the placebo group. However, the investigators noted that the difference in the 2 groups may have been confounded by differences in study participants such as gender and initial HBA_{1c} levels. There were significant differences in the supplemented group as seen by increased glutathione reductase activity, increased plasma protein thiol content, and increased lag time of *ex vivo* copper induced LDL oxidation. Other antioxidant enzymes and oxidation products (TBARS) were not significantly changed with treatment.

4.4. Neurological

A. Alzheimer's Disease

Alzheimer's Disease (AD) is a neurodegenerative disorder, characterized by progressive memory loss and decline in

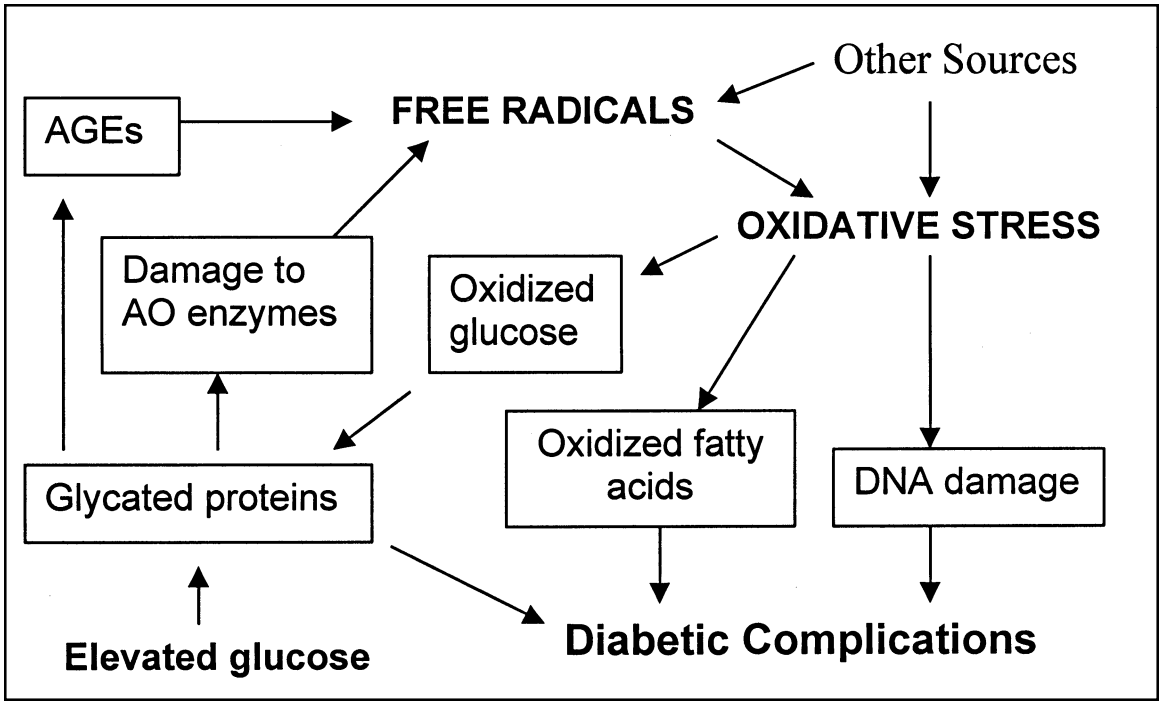

Figure 11 Oxidative stress and diabetic pathology. AGEs = advanced glycation end products, AO = antioxidant. Adapted from McCance et al. 1993.[82]

cognitive skills that affects approximately 4 million people in the United States.[137] A hypothesis implicating free radicals could explain both the heterogeneous presentation of AD and the fact that aging is a significant risk factor for its development. Three key facts support this hypothesis[86]:

1. Neurons are extremely sensitive to free radical attack because their glutathione content is very low, their membranes are high in PUFAs, and brain metabolism requires a substantial amount of oxygen.
2. AD brain lesions are associated with typical free radical damage, e.g. DNA damage, oxidized protein, oxidized lipids, and glycosylated end products.
   - Oxidized DNA is observed in the cerebral cortex of AD patients.[87]
   - Many studies have shown increased lipid peroxidation in the AD brain.[88]
   - Protein oxidation is more marked in AD patients in the regions showing the most pathophysiologic changes.[89]
   - Several studies have identified elevated concentrations of oxidation end products in AD brains, including malondialdehyde, peroxynitrite, carbonyls, advanced glycosylated end products (AGEs), and various enzymes associated with oxidative stress.[90]
3. Metals capable of catalyzing free radical production are naturally present in the brain, e.g. iron, copper, zinc, and aluminum.[91]
   - The concentration of iron is higher in the brains of AD patients.
   - Copper is an important part of antioxidant enzymes, Cu/Zn SOD, and cytochrome-c oxidase and is found in lower concentrations in the brains of AD patients. However, copper ions are powerful promoters of peroxidation and have been linked to the exacerbation of oxidative damage in AD.[92]
   - Zinc induces amyloid formation in humans and there is increasing evidence that high zinc concentrations can mediate neuronal death associated with other brain injuries.[93]
   - Higher levels of aluminum have been measured in the cores of senile plaques in AD. Humans are constantly exposed to this metal through foods, beverages, medications, personal hygiene products, and cookware. It has been proposed that aluminum ions bind to membranes and cause a rearrangement of lipids that lead to propagation of lipid peroxidation.[94] Although aluminum exists only in the trivalent form and is redox-inert, it is possible that it may contribute to lipid peroxidative damage in AD by escalating iron and copper induced free radical damage. An excellent review details the mechanisms that form the hypothesis that aluminum may exacerbate events associated with AD.[95]

Two of the pathological features of AD are neurofibrillary tangles and senile plaques. These plaques are described as localized areas of degenerating and swollen axons, neuritis, and glia surrounding a core of amyloid. These amyloid protein deposits in AD contain truncated products including β-amyloid. Several brain regions show massive neuronal loss in AD.[94] Many *in vitro* studies have confirmed a direct toxic effect of β-amyloid on cultures of neurons. This β-amyloid toxicity is prevented by vitamin E and other antioxidants and mediated by hydrogen peroxide.[96,97]

B. Other Evidence

Isoprostanes, a reliable measurement of *in vivo* lipid peroxidation, are elevated in postmortem frontal and temporal cortex, but not in the cerebellum of patients with AD compared to controls.[98] Similarly, isoprostanes were found to be elevated in the plasma of living AD patients compared to controls.[99] DNA oxidation end products were found to be higher in lymphocytes of AD patients compared to controls.[100] Several studies suggest that abnormal glycosylation of protein is closely related to the presence of lesions found in brains of AD patients.[90] Earlier studies have shown that the coenzyme α-lipoic acid, when fed at 100 mg/Kg body weight, improved memory in aged mice.[101]

4.5. Immune Diseases

Oxidation and generation of free radicals is an essential component of cell-mediated immunity. At physiological levels, free radicals are vital for antigen presentation and cell proliferation. However, at high levels they can decrease immune function.[102] Oxidative stress is detrimental to lymphocytes probably due to lipid peroxidation of PUFAs in the cell membrane and oxidation of plasma LDL that is lymphotoxic. Loss of membrane fluidity in lymphocytes has been correlated to decreased response to immunological challenges.[103]

Antioxidants can improve immune response by controlling the production of free radicals in the cell. Neutrophils kill extracellular pathogens through oxidative bursts. Uptake of vitamin E prior to the oxidative burst protects the neutrophil from destruction by the free radicals generated. Vitamin C is believed to work with vitamin E in this protection. Supplementation with these two antioxidants has been found to normalize the reduced chemotactic and bactericidal activities of neutrophils in compromised individuals.[104]

Manifestation of disease by chronic inflammation is seen in rheumatoid arthritis and inflammatory bowel diseases. Chronic immune induced inflammation has been decreased in animals supplemented with antioxidant enzymes or vitamin E.[105] An example is seen in the animal model of rheumatoid arthritis in which local lipid peroxidation is correlated to inflammation. Administration of antioxidants directly into the joint decreases inflammation.[106]

A. Clinical Trials

Thirty elderly patients, hospitalized for more than 3 months, were supplemented with daily antioxidant vitamins: 8,000 I.U.

vitamin A, 100 mg vitamin C, and 50 mg vitamin E or a placebo. Their cell-mediated immune function was assessed before and after supplementation and improvement was found only in the supplemented group.[107] Another study found significant improvement in the delayed-type hypersensitivity test response in healthy adults supplemented for 30 days with 800 mg/day vitamin E.[108]

B. AIDS

Oxidative stress is believed to contribute to the decline of CD4+ lymphocyte counts and play a significant role in the progression of HIV infection to AIDS. This is understandable since the functioning of lymphocytes is closely linked to their redox potential.[109] It is known that lymphocytes of patients with AIDS are deficient in glutathione which makes them sensitive to oxidative stress. Recently the drug N-acetylcysteine (NAC), a precursor of GSH, has been proposed for use in AIDS.[7]

Plasma levels of vitamin E are known to be lower in HIV-infected patients than in controls. Supplementary vitamin E is significantly associated with slower progression from HIV infection to AIDS and increases CD4+ counts.[110] In AIDS patients plasma levels of zinc, magnesium, selenium, vitamin B_{12}, β-carotene, and other carotenoids are low. The flavonoid, quercetin, inhibits the protein kinase C (PKC)-induced phosphorylation of I-κB that can liberate NF-κB to play a role in viral replication.[109]

4.6. Eye Diseases

Most of the experimental research on the etiology of cataract and advanced macular degeneration (AMD) has focused on vitamins C, E, and carotenoids. Two isomeric carotenoids, lutein and zeaxanthin, have been identified in the rod outer segments of the retina.[111] Their role is hypothesized to be as antioxidants in this highly aerobic tissue where oxidative effects appear to produce the greatest damage.[112] Lutein and zeaxanthin are naturally present in the macula and are found in decreased quantities in the macula of AMD patients.[113]

Animal studies have shown that feeding increased amounts of vitamin C prevented and delayed progress of cataract in guinea pigs and rats. Animal studies have also shown that vitamin E is capable of delaying cataract development in rats and rabbits.[114]

A. Epidemiological Studies

The highest levels of lutein and zeaxanthin in the peripheral region of the macula are reported in one study[113] to be associated with an 82% lower risk of AMD when compared to those with the lowest levels. Several epidemiological studies suggested an association between cataract incidence, macular degeneration, and oxidative stress.[115] Nine of eleven studies showed an inverse association between at least one type of cataract and at least one antioxidant nutrient. Three of five studies showed inverse associations with macular degeneration and at least one antioxidant. Most studies indicate little or no benefit from vitamin supplementation until used for 5 to 9 years. However, significant reduction in cataract risk was observed in women <60 years old after 10 or more years of supplementation with ascorbic acid.[138,139] This finding suggests that we re-evaluate the validity of short term studies of antioxidants that reported minimal or no effects.

The Eye Disease Case Control Study reported an association between decreased serum carotenoids and an increased risk of AMD.[116] In a subsequent study by Seddon et al.,[140] the intake of lutein and zeaxanthin from dietary sources was associated with a significant (58%) reduction in the relative risk of AMD. Goldberg and co-workers[117] reported an inverse association between AMD and consumption of fruits and vegetables high in vitamins A and C from the HANES-I survey.

B. Clinical Trials

The Age Related Eye Disease Study (AREDS) reported a moderate beneficial effect of antioxidants, vitamins, and zinc supplementation in reducing progression to severe AMD.[118] Another AREDS intervention supplemented 4,624 subjects with a daily antioxidant cocktail (500 mg ascorbic acid, 400 IU vitamin E, and 15 mg β-carotene for 6.3 years.[141] They reported no significant effect of antioxidants in the 7 year risk of the development or progression of age-related lens opacities or visual loss. Another trial, the Roche European American Cataract Trial (REACT), studied patients with early ARC supplemented with 750 mg ascorbic acid, 600 mg vitamin E, and 18 mg β-carotene. A significant benefit was reported in the U.S. component but not in the United Kingdom group.[119] There are other trials currently underway to evaluate the effect of antioxidant vitamins on cataracts and AMD, including the Women's Health Study, the Women's Antioxidant Cardiovascular Study, and the Physicians Health Study II.

5. OXIDATIVE STRESS AND THE AGING PROCESS

The aging process is known to be associated with increased oxidative stress, possibly related to loss of antioxidant capacity.[120] Life extension can be achieved by modulating biological and pathological processes. Longevity is determined by the net effect of these two processes including how they affect each other (crosstalk). Currently the only paradigm shown to affect both average and maximum lifespan is caloric restriction. Studies have shown that calorie-restricted rats exhibit increased resistance to oxidative stresses.[121] Perhaps one of the mechanisms by which calorie restriction may increase life span is by increasing resistance to oxidative stresses and enhancing antioxidant defenses. Antioxidant intervention is the focus of one of three general categories of hypotheses based on the current understanding of aging[122]: genetically programmed mechanisms, neuronal-endocrine failures, and oxidatively stressed modifications of cellular lipids, proteins, and DNA.

The Free Radical Hypothesis of Aging (also called the Oxidative Stress Theory of Aging) suggests that age related changes are manifestations of the body's inability to cope with oxidative stress that occurs throughout the lifespan. Normal and pathological aging have been associated with increased sensitivity to free radicals, probably as a result of increases in pro-oxidant mediators and a decrease in antioxidant defense. How this oxidative stress causes the aging process is not known, but it is believed to involve lipid and protein peroxidation, increases in DNA oxidation products, and deficits in calcium regulatory mechanisms that eventually lead to cell death.

Studies conducted over the past years have determined the following changes in mitochondria associated with aging[123]: (1) Some mitochondrial membrane carriers are impaired. Metabolic studies using isolated hepatocytes show that aging affects mitochondrial function by impairing specific inner mitochondrial membrane processes such as malate transport. Ketogenesis from oleate, also dependent on mitochondrial performance, likewise decreased in hepatocytes from old animals. Other mitochondrial functions, such as urea synthesis in hepatocytes, do not decline with age. Since gene expression of the mitochondrial carrier is not changed with aging, post transcriptional modifications appear to be involved in the age related loss of these carriers and is likely to be in response to chronic oxidative stress. (2) Mitochondrial membrane potential declines with age.[124] Because this is the driving force for ATP synthesis, it likely reduces the energy supply from the mitochondria, which in turn may affect protein synthesis. Studies in isolated mitochondria have shown that oxidative stress causes inhibition of respiration, affecting mitochondrial membrane potential. (3) Mitochondria also enlarge with aging. Acute oxidative stress is known to cause mitochondrial swelling.[125] (4) Oxidation of mitochondrial glutathione increases with aging.[126] (5) Age-related mitochondrial DNA damage correlates with glutathione oxidation.[127] (6) Administration of thiol-containing antioxidants protects against age-related glutathione depletion and partially prevents age-related decline in neuromuscular coordination.[128] Administration of other antioxidants, such as gingko biloba extract Egb 761, also prevents mitochondrial DNA damage.[129]

Normally, more than 80% of cellular ATP is derived from oxidative phosphorylation, and the remaining 20% from anaerobic glycolysis. Aged animal tissues have higher glycolytic activity and lower mitochondrial oxidative phosphorylation activity, which is apparently associated with alterations of the structure and function of the mitochondrial F_0F_1-ATP synthase complex. Mitochondria from senescent rats have a decreased F_1 content, which appears to be associated with a decrease in intramitochondrial GSH. Both of these phenomena seem to be related to oxidative damage of the mitochondrial proteins.[130]

5.1. Dietary Implications

A large amount of evidence has accumulated over the years implicating oxidative stress in the physiological changes seen in aging and age-related and neurodegenerative diseases. Consequently, many studies have examined the ability of diet components and specific nutrients to prevent or delay the onset of these changes.[131]

Vitamin E supplementation has been shown to increase the average life span in the rotifer, fruit fly, nematode, and rat.[122] However, studies using the mammal model have shown mixed results. Early studies investigated the effect of chronic vitamin E deficiency in accelerating aging and lipofuscin (an aging pigment) in rats fed either standard rodent chow or a vitamin E deficient diet for 14 months. The effect of vitamin E

Table 2 Summary of epidemiological and clinical trials for vitamin E and cardiovascular disease

Study	Length	Population	Vitamin E	Coronary events	Coronary deaths	Overall mortality
Epidemiological Studies						
Health Prof.[34]	4 years	39,900 M 40–75 y/o	Highest vs. Lowest intake	21% ↓		22% ↓
			≥250 IU supp >2 yrs.	30% ↓		
Nurses' Health[36]	8 years	80,000 F 30–59 y/o	Highest vs. Lowest intake	34% ↓		
			Supplement >2 yrs.		41% ↓	
Finnish Study[39]	14 years	2748 M, 2385 F	Highest vs. Lowest intake		34% ↓ M	
					65% ↓ F	
EPESE[37]	6 years	11,178 M/F 67–105 y/o	Supplement Use		47% ↓	34% ↓
Iowa Women's Health Study[38]	7 years	35,000 F 55–69 y/o	Highest vs. Lowest intake		62% ↓	
Clinical Trials						
Linxian[47]	5 years	29,584 M/F 40–60 y/o	60 IU Vit E/β-carotene/Vit C		10% ↓	9% ↓
ATBC[142]	5–8 years	27,271 male smokers 50–69 y/o	50 IU Vit E	4% ↓	8% ↓	
CHAOS[46]	510 days	2000 M/F with CVD	400–800 IU Vit E	77% ↓	Non-sig effect	Non-sig effect
GISSI[50]	3.5 years	11,324 M/F	300 IU Vit E		20% ↓	35% ↓
HOPE trial[51]	4.5 years	9000 > 55 y/o, high CVD risk.	400 IU Vit E		No effect	
SPACE[143]	17 months	196 dialysis pts.	800 IU Vit E	70% ↓ MI		
SECURE[144]	4.5 yrs.	732 > 55 y/o	400 IU Vit E	No effect		
MRC/BHF[145]	5 yrs.	20,536 M/F	600 IU Vit E 250 mg Vit C 20 mg β Carotene	No effect	No effect	No effect

Table 3 Major on-going antioxidant human clinical trials

Name	Participants	Antioxidant	Primary CVD endpoint
Women's Health Study	40,000 healthy females	Vitamin E 600 IU/day	MI, stroke, and CVD death
SU. VI. MAX Study (Hercbeg et al. 1999)	15,000 French adults	Vitamin E, beta carotene, vitamin E, zinc, and selenium	CVD events
PPP (Progetto di prevenzione pprimaria)	5,500 Italian patients with at least 2 CVD risk factors	Vitamin E 300 IU with aspirin	MI, stroke, and CVD
Physicians Health Study II[86]	15,000 physicians	Beta carotene, vitamin E, vitamin C, multivitamin	Cancer, prostate cancer, CVD, age related eye diseases.
Women's Antioxidant CVD Study	8,000 nurses with established CVD	20 mg beta carotene, 400 IU vitamin E, 100 mg vitamin C	

deficiency was greater than the effect of aging alone on behavioral and histological parameters.[132] A study by Sarter and van der Linde[146] similarly found a higher content of lipofuscin in the hippocampus.

These studies and many others indicate that vitamin E has some protective effects against free radical injuries and age-related degenerative processes. However, they have used only weanling animals because a vitamin E deficiency cannot be produced in adult animals. Therefore, the neuromotor/behavior outcomes may be a developmental phenomena rather than being indicative of an accelerated aging process.

6. CONCLUSIONS

Oxidative stress is clearly associated with a wide range of chronic and acute disease processes. From the standpoint of oxidative stress mechanisms, the hypotheses regarding etiology of heart disease, cancer, neuro-degenerative, and other diseases are certainly plausible and shown to work *in vitro*. However, the real test of efficacy is in the living system.

The epidemiological data generally indicate a benefit of consuming diets that are higher in antioxidant nutrients, specifically diets high in fruits and vegetables. However, there are many inconsistencies in the results. One of the biggest problems is the measurement of long-term nutrient intake and status. To date, only studies that involve nutrient supplements as opposed to dietary intake have been able to accurately assess intake over an extended period of time. In some studies it is not clear whether the benefit is derived from the specific nutrients under study or another food component having health benefits yet to be discovered. Or perhaps, there is a particular combination of antioxidant nutrients that provide protection.

While some epidemiological studies appear to demonstrate clear associations, direct tests of the relationships with clinical trials have not yielded similar results (Table 2). Longer term clinical trials have found minimal to no antioxidant effect on cardiovascular events and mortality. The most convincing evidence of antioxidant effect on cancer prevention involves feeding fruits and vegetables rather than individual antioxidants.

A cluster of healthy behaviors that normally accompany higher intake are difficult to measure and may confound the benefit of specific antioxidant consumption, both dietary and through supplementation. Physical exercise and environmental stress, which generate more ROS, are also difficult to measure in human studies and may further affect the accuracy of both observational and clinical trials. In observational studies, dietary intake and biochemical parameters are measured very infrequently and thus do not provide a clear picture of long term status that is necessary to establish an association. With both observational and clinical trials, the PUFA content of diets is difficult to accurately measure outside of an institutional setting in which actual intake is recorded daily. Another point of concern is that plasma levels of antioxidant vitamins may be an effect of the disease process rather than a measure of dietary intake. This may help to explain why antioxidant therapy has been reported as less effective in smokers and in those with established cardiovascular disease.

Several important questions remain unanswered. For example, are certain subgroups, such as those more oxidatively stressed, more or less receptive to treatment than others? Is there a critical time during which antioxidants can make a significant difference in one's risk of developing a chronic disease? Has the delicate balance between oxidants and antioxidants been tipped by environmental stresses? If so, can the increased intake of dietary antioxidants from food or from supplements correct the imbalance without causing other health concerns?

Clearly, there is a need for more long term, controlled trials in normal and specific chronic disease prone subgroups to study the dose response of each antioxidant nutrient, alone, and in combination with others. Fortunately, such studies are underway, as summarized in Table 3, and others are in the planning stages. However, until we know more about the possible benefits and risks of consuming antioxidants in supplemental concentrations, the best advice is to strive to get more of them by consuming those foods, such as fruits and vegetables, that are excellent dietary sources.

REFERENCES

[1] Harman, D. 1986. In *Free Radicals, Ageing and Degenerative Diseases*. Alan R. Liss, Inc.

[2] Halliwell, B. and Gutteridge, J.M.C. 1999a. Ch. 1, Antioxidant Defences. In *Free Radicals in Biology and Medicine*, 3rd Ed. Oxford University Press-New York.

[3] Halliwell, B. 1994. Free radicals and antioxidants: A personal view. Nutrition Reviews, **52**:253–265.
[4] Turrens, J. 1997. Superoxide production by the mitochondrial respiratory chain. *Biosci. Rep.*, **17**:3–8.
[5] Shigenaga, M., Hagen, T., and Ames, B. 1994. Oxidative damage and mitochondrial decay in ageing. *Proc. Natl. Acad. Sci. USA* **91**:10771–10778.
[6] Borg, D.C. 1993. Oxygen free radicals and tissue injury. In *Oxygen Free Radicals in Tissue Damage* (Ch 2) Ed. Tarr and Samson, Birkhauser Boston.
[7] Roberford, M. and P.B. Calderon, 1995. (Ch. 5) *Free Radicals and Oxidation Phenomena in Biological Systems*, Dekker, New York.
[8] Punchard, N.A. and F. Kelly, 1997. *Free Radicals-A Practical Approach*, Oxford University Press, New York.
[9] Chow, C.K. 1993. Vitamin E and cigarette smoking-induced oxidative damage. (Ch. 48) In *Vitamin E in Health and Disease.* Ed. Packer and Fuchs.
[10] Niki, E. 1997. Free radicals in chemistry and biochemistry. In *Food and Free Radicals*, p.2. Ed. Hiramatsu et al., Plenum Press, New York.
[11] Mates, J.M., Perez-Gomez, C., and Nunez De Castro, I. 1999. Antioxidant enzymes and human diseases. *Clinical Biochemistry*, **32**:595–603.
[12] Visioli, F. 1998. Free radical scavenging properties of olive oil polyphenols. *Biochem Biophys Res Comm.*, **247**:60–64.
[13] Halliwell, B. and Gutteridge, J.M.C. 1999b. Ch. 4, Oxidative Stress: Adaptation, Damage, Repair and Death. In *Free Radicals in Biology and Medicine*, 3rd Ed. Oxford University Press, New York.
[14] Haddad, J. 2002. Antioxidant and prooxidant mechanisms in the regulation of redox(y)-sensitive transcription factors. *Cellular Signaling*, **14**:879–897.
[15] Moreno, J.J. and Mitjavila, M.T. 2003. The degree of unsaturation of dietary fatty acids and the development of atherosclerosis (review). *J. Nutr. Biochem.*, **14**:182–195.
[16] Mashima, R., Witting, P.K., and Stocker, R., 2001. Oxidants and antioxidants in atherosclerosis. *Curr. Opin. Lipidol.*, **12**:411–418.
[17] Steinberg, D. 1993. Oxidative modification of LDL and atherosclerosis. *Circulation*, **95**:1062–1071.
[18] Westhuysen, J. 1997. The oxidation hypothesis of atherosclerosis: An update.*Ann. Clin. Lab. Sci.*, **27**:1–10.
[19] Frostegard, J., Hagerstrand, A., Gidlund M., and Nilsson, J. 1991. Biologically modified low density lipoprotein increases the adhesive properties of endothelial cells. *Atherosclerosis*, **90**:119–126.
[20] Henriksen, T., Mahoney, E.M., and Steinberg, D. 1981. Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: Recognition of receptors for acetylated LDL. *Proc. Natl. Acad. Sci. USA*, **78**:6499–6503.
[21] Quinn, M.T., Parthasarathy, S., Steinberg, D, and Fong, L.G., 1987. Oxidatively modified LDL: A potential role in recruitment and retention of monocyte/macrophages during atherogenesis. *Proc. Natl. Acad. Sci. USA*, **84**:2995–2998.
[22] Cathcart, M.K., Morel, D.W., and Chisholm, G.M. III. 1985. Monocytes and neutrophils oxidize LDL making it cytotoxic. *J. Leukoc. Biol.*, **38**:341–350.
[23] Pratico, D., Iuliano, L., Mauriello, A. et al. 1997. Localization of distinct F2-isoprostanes in human atherosclerotic lesions. *J. Clin. Invest.*, **100**:2028–2034.
[24] Holvoet, P., Perez, G., Zhao, Z., et al. 1995. Malonaldehyde-modified low density lipoprotein in patients with atherosclerotic disease. *J. Clin. Invest.*, **95**:2611–2619.
[25] Yla-Herttuala, Palinski, W., Rosenfeld, M.E. et al 1989. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J. Clin. Invest.*, **84**:1086–1095.
[26] Palinski, W., Rosenfeld, M.E., Yla-Herttuala, S., Gurtner, G.C., Socher, S.S., Butler, S.W., Parthasarathy, S., Carew, T.E., Steinberg, D., and Witztum, J.L. 1989. Low-density lipoprotein undergoes oxidative modification in vivo. *Proc. Natl. Acad. Sci.*, **86**:1372–1376.
[27] Salonen, T., Yla-Herttulal, S., and Yamamoto, R. 1992. Autoantibodies against oxidized LDL and progression of carotid atherosclerosis. *Lancet*, **339**:883–887.
[28] Esterbauer, H., Dieber-Rothender, M. Striegl, G., and Waeg, G. 1991. Role of vitamin E in preventing the oxidation of low-density lipoprotein. *AJCN* **53 S**:314S–321S.
[29] Arora, A., Byrem, T., Nair, M., and Strasburg, G. 2000. Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. *Arch. Biochem. Biophys.*, **373**:102–109.
[30] Halliwell, B. and Gutteridge, J. 1992. Biologically relevant metal ion dependent $OH^{\cdot}$ generation. An update. FEBS Lett. **307**:108–112.
[31] Yoshikawa, T., Naito, Y., and Kondo, M. 1997. Free radicals and disease Ch.2, In, *Food and Free Radicals*, Eds. Hiramatsu, M., Yoshikawa, T., Inoue, M. Plenum Press, New York.
[32] Roberts, L. and Morrow, J., 2000. Measurement of F2-Isoprostanes as an index of oxidative stress in vivo. *Free Rad. Biol. Med.*, **28**:505–513.
[33] Gey, K.F., Puska, P., Jordan, P., and Moser, U.K. 1991. Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. *ACJN*, **53**:326–34S.
[34] Rimm, E.B., Stampfer, M.J., Ascherio, A., et al. 1993. Vitamin E consumption and risk of coronary heart disease in men. *N. Engl. J. Med.*, **328**:1444–1449.
[35] Rimm, E.B., Katan, M.B., Ascherio, A., Stampfer, M.J., and Willett, W.C. 1996. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Annals. Intern. Med.*, **125**:384–389.
[36] Stampfer, M.J., Hennekens, C.H., Manson, J.E., Colditz, G.A., Rosner, B., and Willett, W.C. 1993. Vitamin E consumption and risk of coronary disease in women. *New Engl. J. Med.*, **328**:1444–1449.
[37] Losonczy, K.G., Harris, T.B., and Havlik, R.J. 1996. Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: The established populations for epidemiologic studies of the elderly. *Am. J. Clin. Nutr.*, **64**:190–196.
[38] Kushi, L.H., Folsom, A.R., Prineas, R.J. et al. 1996. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. *N. Engl. J. Med.*, **334**:1156–1162.
[39] Knekt, P., Reunanen, A., Jarvinen, R., Seppanen, R., Heliovaara, M., and Aromaa, A. 1994. Antioxidant vitamin intake and coronary mortality in a longitudinal population study. *Am. J. Epidemiol.*, **139**:1180–1189.
[40] Hertog, M.G., Feskens, E.J.M., Hollman, P.C.H., Katan, M.B., and Krombout, D. 1993. Dietary antioxidant flavonoids and risk of coronary heart disease. The Zutphen Elderly Study. *Lancet*, **342**:1007–1011.
[41] Hertog, M.G.L., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., Giampaoli, S., Jansen, A., Menotti, A., Nedeljkovic, S., Pekkarinen, M., Simic, B.S., Toshima, H., Feskens, E.J.M., Hollman, P.C.H., and Katan, M.B. 1995. Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. *Arch. Intern. Med.*, **155**:381–386.
[42] Knekt, P., Jarvinen, R., Reunanen, A., and Maatela, J. 1996. Flavonoid intake and coronary mortality in Finland: A cohort study. *Brit. Med. J.*, **312**:478–481.
[43] Renaud, S. and de Lorgeril, M. 1992. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*, **339**:1523–1526.
[44] Frankel, E.N. 1995. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human LDL. *J. Agric. Food Chem.*, **43**:890.
[45] Joshipura, K.J., Hu, F.B., and Manson, J.E. 2001. The effect of fruit and vegetable intake on risk for coronary heart disease. *Ann. Intern. Med.*, **134**:1106–1114.
[46] Stephens, N.G., Parsons, A., Schofield, P.M., Kelly, F., Cheeseman, K., Mitchinson, M.J., and Brown, M.J. 1996. Randomized controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS) *Lancet*, **347**:781–786.
[47] Blot, W.J., Li, J.Y., Taylor, P.R., Guo, W., Dawsey, S., Wang, G.O., Yang, C.S., Zheng, S.F., Gail, M., Li, G.Y., Yu, Y., Liu, B.Q., Tangrea, J., Sun, Y.H., Liu, F., Fraumeni, J.F., Zhang, Y.H., and Li, B. 1993. Nutrition intervention in trials in Linxian, China: Supplementation with

specific vitamin/mineral combinations, cancer incidence and disease specific mortality in the general population. *J. Natl. Cancer. Inst*., **85**:1483–1492.

[48] Alpha-tocopherol, Beta- Carotene Cancer Prevention Study Group. 1994. The effect of Vitamin E and Beta-Carotene on the Incidence of Lung Cancer and Other Cancers in Male Smokers. *New Engl. J. Med*., **330**:1029–1035.

[49] Omenn, G.S., Goodman, G.E., Thornquist, M.D., Balmes, J., Cullen, M.R., Glass, A. et al. 1996. Effects of a combination of β-Carotene and vitamin A on lung cancer and cardiovascular disease. *NEJM*, **334**:1150.

[50] Marchioli, R. 1999. Antioxidant vitamins and prevention of cardiovascular disease: Laboratory, epidemiological and clinical trial data. *Pharmacological Research*, **40**:3 227–238.

[51] Yusuf, S. 2000. Vitamin E Supplementation and Cardiovascular Events in High-Risk Patients. *New England Journal of Medicine*, **342**:1917–1918.

[52] Hodis, H., Mack W., LaBree, L., Mahrer, P., Sevanian, A., Liu, C., Liu, C., Hwang, J., Selzer, R., and Azen, S. 2002. Alpha-tocopherol supplementation in healthy individuals reduces low density lipoprotein oxidation but not atherosclerosis: The Vitamin E Atherosclerosis Prevention Study (VEAPS). *Circulation*, **106**:1453–1459.

[53] Heart Protection Study Collaborative Group, 2002. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: A randomized placebo-controlled trial. *Lancet*, **360**: 23–33.

[54] American Cancer Society Cancer. *Facts and Figures 1996*, Atlanta GA.

[55] McLarty, J.W. 1997. Antioxidants and Cancer: The Epidemiologic Evidence, (Ch.5) In *Antioxidants and Disease Prevention*.

[56] Loo, G. 2003. Redox-sensitive mechanisms of phytochemical-mediated inhibition of cancer cell proliferation (Review). *J. Nutr. Biochem*., **14**:64–73.

[57] Watson, R.R. and Leonard, T.K. 1986. Selenium and vitamins A, E, and C: Nutrients with cancer prevention properties. *JADA*, **86**:505–510.

[58] Cross, C.E. 1987. Oxygen radicals and human disease. *Ann. Intern. Med*., **107**:526–545.

[59] Totter, T.R. 1980. Spontaneous cancer and its possible relationship to O_2 metabolism. *Proc. Natl. Acad. Sci. USA*, **77**:1763.

[60] Loft, S. and Poulsen, H. 1996. Cancer risk and oxidative DNA damage in men. *J. Mol. Med*., **74**:297.

[61] Cerutti, P. 1994. Oxy-radicals and cancer. *Lancet*, **344**:862–865.

[62] Shigenaga, M., Park, J., Cundy, K., Gimeno, C., and Ames, B., 1990. In vivo oxidative DNA damage; Measurement of 8-hydroxy-2-deoxyguanosine in DNA and urine by high-performance liquid chromatography with electrochemical detection. *Methods Enzymol*., **186**:521–530.

[63] Fisher-Nielsen, A., Loft, S., and Jensen, K.G. 1993. Effect of ascorbate and 5-aminosalicylic acid on light-induced –hydroxydeoxyguanosine formation in V79 Chinese hamster cells. *Carcinogenesis*, **14**(11): 2431–2433.

[64] Haegele, A.D., Briggs, S.P., and Thompson, H.J., 1994. Antioxidant status and dietary lipid unsaturation modulate oxidative DNA. *Free Radic. Biol. Med*., Jan.**16**(1):111–115.

[65] Djuric, Z., Heilbrun, L.K., Reading, B.A., Boomer, A., Valeriote, F.A., and Martino, S. 1991. Effects of low-fat diet on levels of oxidative damage to DNA in human peripheral nucleated blood cells. *J. Natl. Cancer Inst*., **83**:766–769.

[66] Block, G., Patterson, B.H., and Subar, A.F. 1992. Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. *Nutr. Cancer* **18**:1–29.

[67] Block, G. 1999. Emerging roles in nutrition in chronic disease prevention: A look at the data with an emphasis on Vitamin C (Ch. 3) In *Antioxidant Food Supplements in Human Health*.

[68] Howe, G.R., Hirohata, T., Hislop T.G., et al. 1990. Dietary factors and risk of breast cancer: Combined analysis of 12 case control studies. *J. Natl. Cancer Inst*., **82**:561–569.

[69] Van Poppel, G. and Goldbohm, R.A. 1995. Epidemiologic evidence for β-carotene and cancer prevention. *Am. J. Clin. Nutr*., **62**:1393–402S.

[70] Giovannucci, E. 1999. Tomatoes, tomato-based products, lycopene, and cancer: Review of the epidemiological literature. *J. Natl. Cancer Inst*., **91**:317–332.

[71] Paolini, M., Abdel-Rahman, S.Z., Sappone, A., Pedulli, G.F., Perocco, P., Cantelli-Forti, G., and Legator, M.S., 2003. β-Carotene: A cancer chemopreventive agent or a co-carcinogen? *Mutation Research*, **543**:195–200.

[72] Combs, G.F. 1997. Selenium and cancer prevention. In *Antioxidants and Disease Prevention*. Ed. Garewal, CRC Press, Boca Raton, FL.

[73] Willis, M.S. and Wians, F.H. 2003. The role of nutrition in preventing prostate cancer: A review of the proposed mechanism of action of various dietary substances. *Clinica. Chimica. Acta*., **330**:57–83.

[74] Greenberg, E.R., Baron, J.A., Tosteson, T.D., Freeman, D.H., Jr., Beck, G.J., Bond, J.H., Colacchio, T.A., Coller, J.A., Frankl, H.D., Haile, R.W. et al. 1994. A clinical trial of antioxidant vitamins to prevent colorectal adenoma. Polyp Prevention Study Group. *NEJM*., **331**: 141–147.

[75] Baron, J.A., Cole, B.F., Mott, L., Haile, R., Grau, M., Church, T.R., Beck, G.J., and Greenberg, E.R. 2003. Neoplastic and antineoplastic effects of β-carotene on colorectal adenoma recurrence: Results of a randomized trial. *Journal of Cancer Research*, **95**(10):717–722.

[76] Thompson, H.J., Heimendinger, J., Haegele, A., Sedlacek, S.M., Gillette, C., O'Neill, C., and Wolfe, P.C. 1999. Effect of increased vegetable and fruit consumption on markers of oxidation. *Carcinogenesis*, Dec. **20**(12):2261–2266.

[77] Drisko, J.A., Chapman, J., and Hunter, V.J. 2003. The use of antioxidant therapies during chemotherapy. *Gynecologic Oncology*, **88**:434–439.

[78] Maritim, A.C., Sanders, R.A., and Watkins, J.B. 2003. Diabetes, oxidative stress and antioxidants. *J. Biochem. Molecular Toxicology*, **17**(1): 24–37.

[79] Lyons, T.J. 1991. Oxidized LDL: A role in the pathogenesis of atherosclerosis in diabetes. *Diabet. Med*., **8**:411–419.

[80] Baynes, J.W. 1991. Role of oxidative stress in development of complications of diabetes. *Diabetes*, **40**:405–412.

[81] Mezzetti, A., Cipollone, F., and Cuccurullo, F. 2000. Oxidative stress and cardiovascular complications in diabetes: Isoprostanes as new markers on an old paradigm, *Cardiovascular Research*, **47**: 475–488.

[82] McCance, D.R., Dyer, D.G., Dunn, J.A., Bailie, K.E., Thorpe, S.R., Bayes, J.W., and Lyons, T.J. 1993. Maillard reaction products and their relation to complications in insulin-dependent diabetes mellitus. *J. Clin. Invest*., **91**:22470–22478.

[83] Halliwell, B. and Gutteridge, J.M.C. 1999c. Ch. 9, Free Radicals, other Reactive Species and Disease. In *Free Radicals in Biology and Medicine*, 3rd Ed. Oxford University Press, New York.

[84] Lean, M.E.J., Noroozi, M., Kelly, I., Burns, J., Talwar, D., Sattar, N., and Crozier, A. 1999. Dietary flavonols protect diabetic human lymphocytes against oxidative damage to DNA. *Diabetes*, **48**:176–181.

[85] Keenoy, B., Vertommen, J., and Leeuw, I. 1999. The effect of flavonoid treatment on the glycation and antioxidant status in Type I diabetic patients. *Diab. Nutr. Metab*., **12**:256–263.

[86] Christen, Y. 2000. Oxidative stress in Alzheimer's Disease. *Am J. Clin. Nutr*., **71**(2):621–29S.

[87] Mullaart, E., Boerrigter, M.E., and Ravid R. 1990. Increased levels of DNA breaks in cerebral cortex of Alzheimer's disease patients. *Neurobiol. Ag*., **11**:169–173.

[88] Markesbery, W.R. and Levell, M.A. 1998. Four-hydroxynonenal, a products of lipid peroxidation, is increased in the brain in Alzheimer's disease. *Neurobiol. Ag*., **19**:33–36.

[89] Smith, M.A., Perry, G., and Richey. P.L. 1996. Oxidative damage in Alzheimer's. *Nature*, **382**:120–121.

[90] Pratico, D. and Delanty, N. 2000. Oxidative injury in diseases of the central nervous system: Focus on Alzheimer's Disease. *Am. J. Med*., **109**:577–585.

[91] Lovell, M.A., Robertson, J.D., Teesdale, W.J., Campbell, J.L., Markesbery, W.R. 1998. Copper, iron and zinc in Alzheimer's disease senile plaques. *J. Neurol. Sci.,* **158**(1):47–52.

[92] Becaria, A., Bondy, S.C., and Campbell, A. 2003. Aluminum and copper interact in the promotion of oxidative but not inflammatory events: Implications for Alzheimer's disease. *J. Alzheimers Dis*., **5**(1): 31–38.

[93] Danscher, G., Jensen, K.B., Frederickson, C.J., Kemp, K., Andreasen, A., Juhl, S., Stoltenberg, M., and Ravid, R. 1997. Increased amount of zinc in the hippocampus and amygdala of Alzheimer's diseased brains: A proton-induced X-ray emission spectroscopic analysis of cryostat sections from autopsy material. *J. Neurosci. Methods*, **76**(1):53–9.

[94] Halliwell, B. and Gutteridge, J.M.C. 1999d. Ch. 8, Free Radicals, Reactive Species and Toxicology. In *Free Radicals in Biology and Medicine*, 3rd Ed. Oxford University Press, New York.

[95] Campbell, A. 2002. The potential role of aluminum in Alzheimer's disease. *Nephrol Dial Transplant*, **17**(Suppl 2):17–20.

[96] Behl, C., Davis, J., Cole, G.M., and Schubert, D. 1992. Vitamin E protects nerve cells from amyloid B protein toxicity. *Biochem. Res. Comm*., **186**:944–950.

[97] Grundman, M. 2000. Vitamin E and Alzheimer disease: The basis for additional clinical trials. *AJCN*, **71S**:660S–666S.

[98] Pratico, D., Lee V.M.Y., and Trojanowski, J.Q., 1998. Increased F2-iosprostanes in Alzheimer's disease. Evidence for enhanced lipid peroxidation in vivo. *FASEB. J*., **12**:1777–1783.

[99] Waddington, E., Croft, K., and Clarnette, R. 1999. Plasma F2-isoprostane levels are increased in Alzheimer's disease: Evidence of increased oxidative stress in vivo. *Alzheimer's Report*., **2**:277–282.

[100] Wade, R., Hirai, K., Perry, G., and Smith, M.A. 1998. Accumulation of 8-hydroxy-guanosine in neuronal cytoplasm indicates mitochondrial damage and radical production are early features of Alzheimer's disease. *J. Neuropathol. Exp. Neurol*., **57**:511.

[101] Stoll, S., Rostoch, A., Bartsch, R., Lorn, E., Meichelboch, A., and Muller, W.E. 1994. The potent free radical scavenger alpha-lipoic acid improves cognition in rodents. *Annals of the New York Academy of Sciences*, **717**:122–128.

[102] Hughes, D.A. 1999. Effects of dietary antioxidants on the immune function of middle-aged adults, *Proc. Nutr. Soc*., **58**:79–84.

[103] Douziech, N., Seres, I., Larbi, A., Szikszay, E., Roy, P., Arcand, M., Dupuis, G., and Fulop T. 2002. Modulation of lumen lymphocyte proliferative response with aging. *Experimental Gerontology*, **37**: 369–387.

[104] Grimble, R.F. 1998. Modification of inflammatory aspects of immune function by nutrients. *Nutrition Research*, **18**:1297–1317.

[105] Inserra, P.F., Ardestani, S., and Watson, R. 1997. Antioxidants and immune function. In *Antioxidants and Disease Prevention*. ed. Garewel.

[106] Sies, H. 1985. *Oxidative Stress*, Academic Press, London.

[107] Penn, N.D., Purkins, L., Kelleher, J., Heatley, R.V., Mascie-Taylor, B.H., and Belfield, P.W., 1991. The effect of dietary supplementation with vitamins A, C, and E on cell-mediated immune function in elderly long-stay patients: A randomized controlled trial. *Ageing*, **20**: 169–174.

[108] Meydani, S.N., Barklund, M.P., Liu, S., Meydani, M., Miller, R.A., Cannon, J.G., Morrow, F.D., Rocklin, R., and Blumberg, J.B. 1990. Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. *Am. J. Clin. Nutr*., **52**:557–563.

[109] Zhang, Z., Inserra, P.F., and Watson, R.R. 1997. Antioxidants and AIDS, In *Antioxidants and Disease Prevention* Garewal, H.S. Ed., CRC Press, Boca Raton.

[110] Abrams, B., Duncan, D., and Hertz-Picciotto, I. 1993. A prospective study of dietary intake and acquired immune deficiency syndrome in HIV-seropositive homosexual men. *J. AIDS*, **6**:949.

[111] Rapp, L.M., Maple, S.S., and Choi, J.H. 2000. Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina. *Invest. Ophthalmol. Vis. Sci*., **41**: 1200–1209.

[112] Krinsky, N.I., Landrum, J.T., and Bone, R.A. 2003. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. *Annu. Rev. Nutr*., **23**:171–201.

[113] Bone, R.A., Landrum, J.T., Mayne, S.T., Gomez, C.M., Tibor, S.E., and Twaroska, E.E. 2001. Macular pigment in donor eyes with and without AMD: A case-control study. *Invest. Ophthalmol. Vis. Sci*., **42**: 235–240.

[114] Jacques, P.F. 1999. The potential preventive effects of vitamins for cataract and age-related macular degeneration. *Int. J. Vitam. Nutr. Res*., **69**:198–205.

[115] Franks, R., Amin, R., and Puklin, J. 1999. Antioxidant enzymes in the macular retinal pigment epithelium of eyes with neovascular age-related macular degeneration, *American Journal of Ophthalmology*, **127**: 694–709.

[116] Eye Disease Case-Control Study Group. 1992. Risk factors for neovascular age-related macular degeneration. *Arch. Ophthalmol*., **110**:1701–1708.

[117] Goldberg, J., Flowerdew, G., Smith, E., et al. 1988. Factors associated with age-related macular degeneration. An analysis of data from the first National Health and Nutrition Examination Survey. *Am. J. Epidemiol*., **128**:700–710.

[118] Hyman, L. and Neborsky, R. 2002. Risk factors for age-related macular degeneration: An update. *Current Opinion in Ophthalmology*, **13**:171–175.

[119] Chylack, L.T., Brown, N.P., Bron, A., Hurst, M., Kopcke, W. et al. 2002. The Roche European American Cataract Trial (REACT): A randomized clinical trial to investigate the efficacy of an oral antioxidant micronutrient mixture to slow progression of age-related cataract. *Ophthalmic Epidemiol*., **9**:49–80.

[120] Loo, B., Bachschmid, M., Spitzer, V., Brey, L., Ullrich, V., and Luscher, T.F. 2003. Decreased plasma and tissue levels of vitamin C in a rat model of aging: Implications for antioxidant defense. *Biochem. and Biophys. Res. Comm*., **303**:483–487.

[121] Sohal, R.S. and Weindruch, R. 1996. Oxidative stress, caloric restriction, and aging. *Science*, **273**:59–63.

[122] Yu, B.P. 1999. Approaches to anti-aging intervention: The promise and the uncertainties. *Mechanisms in Ageing and Development*, **111**:73–87.

[123] Vina, J., Sastre, J., Pallardo, F.V., and Lloret, A. 1999. Involvement of mitochondria in oxidative stress in aging of intact cells. In *Natural Antioxidants and Anti-carcinogens in Nutrition, Health and Disease*. Kumpulainen, J.T. and Salonen, J.T. Eds. Royal Society of Chemistry, Cambridge, U.K.

[124] Sastre, J., Pallardo, F., Pla, R., Pellin, A., Juan, G., O'Conner, E., Estrela, J.M., Miquel, J., and Vina, J. 1996. Aging of the liver: Age-asociated mitochondrial damage in intact hepatocytes. *Hepatology*, **24**: 1199–1205.

[125] Bernardi, P. 1991. Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by the proton electrochemical gradient. *J. Biol. Chem*., **267**:8834–8839.

[126] Hazelton, G.A. and Lang C.A. 1980. Glutathione contents of tissues in the aging mouse. *Biochem J*., **188**:25–30.

[127] Garcia, J., Millan, A., Pla, R., Bruseghini, L., Esteras, A, Pallardo, F.V., Sastre, J., and Vina, J. 1996. Mitochondrial glutathione oxidation correlates with age associated oxidative damage to mitochondrial DNA. *FASEB J*., **10**:333–338.

[128] Vina, J., Sastre, J., Anton, V., Bruseghini. L., Esteras. A., and Asensi, M. 1992. Effect of aging on glutathione metabolism. Protection by antioxidants. In *Free radicals and aging*. Emerit. I. and Chance. B. Eds., Birkhauser Verlag. Basel.

[129] Droy-Lefaix, M.T. and Packer, L. 1999. Antioxidant properties of ginkgo biloba extract: Egb 761. In *Antioxidant Food Supplements in Human Health* Ed. Packer, Hiramatsu, Yoshikawa.

[130] Guerrieri, F., Pellecchia, G., and Papa S. 1998. Ch. 11. Reactive oxygen species (ROS) and alternation of F_0F_1-ATP synthase in aging and liver regeneration. In *Free Radicals, Oxidative Stress, and Antioxidants. Pathological and Physiological Significance*. Ed. Ozben, T., Plenum Press, New York.

[131] Cantuti-Castelvetri, I., Shukitt-Hale, B., and Joseph, J.A. 2000. Neurobehavioral aspects of aging. *Int. J. Devl. Neuroscience*, **18**:367–381.

[132] Lal, H., Pogacar, S., Daly, P.R., and Puri, S.K. 1973. Behavioral and neuropathological manifestations of nutritionally induced central nervous system "aging" in the rat. *Prog. Brain Res.*, **40**:129–140.

[133] Willcox, J.K., Catignani, G.L., and Lazarus, S. 2003. Tomatoes and cardiovascular health. *Critical Reviews in Food Science and Nutrition.*, **43**(1):1–18.

[134] Asplund, K. 2002. Antioxidant vitamins in the prevention of cardiovascular disease: A systematic review. *J. Intern. Med.*, **251**(5):372–392.

[135] Ziegler, R.G., Mayne, S.T., and Swanson, C.A. 1996. Nutrition and lung cancer. *Cancer Causes Control*, **7**(1):157–177.

[136] Collins, A.R., Raslova, K., Somorovska, M., Petrovska, H., Ondrusova, A., Vohnout, B., Fabry, R., and Dusinska, M. 1998. DNA damage in diabetes: Correlation with a clinical marker. *Free Radic. Biol. Med.*, **25**(3):373–377.

[137] Pratico, D. 2002. Alzheimer's disease and oxygen radicals: New insights. *Biochem. Pharm.*, **63**:563–567.

[138] Taylor, A. 1993. Cataract: Relationships between nutrition and oxidation. *J. Am. Cell. Nutr.*, **12**:138.

[139] Chasan-Taber, L., Willett, W.C., Seddon, J.M., Stampfer, M.J., Rosner, B., Colditz, G.A., and Hankinson, S.E. 1999. A prospective study of vitamin supplement intake and cataract extraction among U.S. women. *Epidemiology*, **10**(6):679–684.

[140] Seddon, J.M., Ajani, U.A., Sperduto, R.D., Hiller, R., Blair, N., Burton, T.C., Farber, M.D., Gragoudas, E.S., Haller, J., Miller, D.T., et al. 1994. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA*, **272**(18):1413–1420.

[141] AREDS Research Group. 2001. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age related macular degeneration and vision loss: AREDS report no. 8. *Arch. Ophthalmol.*, **119**(10):1417–1436.

[142] Alpha-tocopherol, Beta-Carotene Cancer Prevention Study Group. 1994. The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. *New Engl. J. Med.*, **330**:1029–1035.

[143] Boaz, M., Smetana, S., Weinstein, T., et al. 2000. Secondary prevention with antioxidants of cardiovascular disease in end-stage renal disease (SPACE): Randomised placebo-controlled trial. *Lancet.*, **356**(9237):1213–1218.

[144] Lonn, E., Yusuf, S., Dzavik, V., Doris, C., Yi, Q., Smith, S., Moore-Cox, A., Bosch, J., Riley, W., Teo, K., SECURE Investigators. 2001. Effects of ramipril and vitamin E on atherosclerosis: The study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE). *Circulation*, **103**(7):919–925.

[145] Heart Protection Study Collaborative Group. 2002. MRC/BHF heart protection study of antioxidant vitamin supplementation in 20,536 high-risk individuals: A randomised placebo-controlled trial. *Lancet*, **360**(9326):23–33.

[146] Sarter, M., and van der Linde, A. 1987. Vitamin E deprivation in rats: Some behavioral and histochemical observations. *Neurobiol. Aging*, **8**(4):297–307.

[147] Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases, 2002.