Antioxidants and Healthy Life
(Current Researches)

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I. INTRODUCTION

Antioxidants

An **antioxidant** is a chemical that reduces the rate of particular **oxidation** reactions in a specific context, where **oxidation reactions** are **chemical reactions** that involve the transfer of **electrons** from a substance to an **oxidizing agent**, this generally results in different chemicals to the original ones.

Antioxidants are particularly important in the context of **organic chemistry** and **biology**. All living organisms maintain a reducing environment inside their cells, all cells contain complex systems of antioxidants to prevent chemical damage to the cells' components by oxidation. These antioxidants include **glutathione** and **ascorbic acid** and are substrates for enzymes such as **peroxidases** and **oxidoreductases.** *[From Wikipedia, the free encyclopedia]*

Antioxidants are widely used as ingredients in **dietary supplements** used for health purposes such as preventing cancer and heart disease. Studies have suggested antioxidant supplements has benefits for health, but several large **clinical trials** did not demonstrate a definite benefit for the formulations tested, and excess supplementation may even be harmful.

Dietary supplementation has few specific antioxidants compared to a broad diet rich in **phytonutrients,** which will yield thousands of different **polyphenol** antioxidants available for metabolism.

What is an Antioxidant?

We have heard about the health benefits of antioxidants, but many of us do not know what an antioxidant is -- and how they actually work?

Antioxidants are our friends, they are dietary substances including some nutrients such as beta carotene, vitamins C and E and selenium, that can prevent damage to our body cells or repair damage that has been done.

Antioxidants are the **knights in shining armour** that subjugate the attack of free radicals in the body, the hazardous molecules that damage cells and procure aging and disease. Though antioxidants are produced naturally in the body, these decline with age, hence there is an increasing need to acquire them from the foods in our diet.

Antioxidants work by significantly slowing or preventing the oxidative -- or damage from oxygen -- process caused by substances called free radicals that can lead to cell dysfunction and the onset of problems like heart disease and diabetes. Antioxidants may also improve the immune function and perhaps lower our risk of infection and cancer. In our body, the antioxidant process is similar to stopping an apple from

browning. Once you cut an apple, it begins to brown, but if you dip it in orange juice, which contains vitamin C, it stays white.

An eating plan containing plenty of fruits and vegetables, whole grains and nuts can supply all the antioxidants your body needs. From warding off heart disease to slowing degeneration of the brain and eyes, talk of the health benefits of antioxidants are quite common today. Antioxidants work by neutralizing highly reactive, destructive compounds called free radicals.

The term antioxidant (also "antioxygen") originally referred specifically to a chemical that prevented the consumption of molecular oxygen. In the 19th and early 20th century, antioxidants were the subject of extensive research in **industrial processes** such as the **corrosion of metals, explosives, the vulcanization** of rubber, and the **knocking** of fuels **in internal combustion engines**.[1]

Early nutrition researchers focused on the use of antioxidants for preventing the oxidation of **unsaturated fats,** the cause of **rancidity.** Antioxidant activity could be measured simply by placing the fat in a closed glass container with oxygen and observing the rate of oxygen consumption. However, it was the identification of **vitamins A, C, and E** as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in biology.

The possible **mechanisms of action** of antioxidants were first explored thoroughly by Moreau and Dufraisse (1926), who recognized that a substance with anti-oxidative activity is likely to be one that is itself a target for oxidation. Research into how vitamin E prevents the process of **lipid peroxidation** led to the current understanding of antioxidants as **reducing agents** that break oxidative chain reactions, often by scavenging **reactive oxygen species** before they can cause damage to the cells.[2]

The Antioxidant Process

Antioxidants block the process of oxidation by neutralizing free radicals. In doing so, the antioxidants themselves become oxidized. That is why there is a constant need to replenish our antioxidant resources. How these work can be classified in one of two ways:

Chain-breaking

- When a free radical releases or steals an electron, a second radical is formed. This molecule then turns around and does the same thing to a third molecule, continuing to generate more unstable products. The process continues until termination occurs -- either the radical is stabilized by a chain-breaking antioxidant such as beta-carotene and vitamins C and E, or it simply decays into a harmless product.

Preventive –

Antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase prevent oxidation by reducing the rate of chain initiation. That is, by scavenging initiating radicals, such antioxidants can thwart an oxidation chain from ever setting in motion. They can also prevent oxidation by stabilizing transition metal radicals such as copper and iron.

The effectiveness of any given antioxidant in the body depends on which free radical is involved, how and where it is generated, and where the target of damage is. Thus, while in one particular system an antioxidant may protect against free radicals, in other systems it could have no effect at all. Or, in certain circumstances, an antioxidant may even act as a "pro-oxidant" that generates toxic oxygen species.

Free Radicals

Free radical production is actually a normal part of life, part of the equation of simply breathing in oxygen. Usually, the body's natural defence systems neutralize free radicals that develop, rendering them harmless. However, environmental assaults on the body, such as UV-radiation, pollutants and alcohol, can overpower the body's ability to neutralize free radicals, allowing them to cause damage to the structure and function of the body's cells. There is good evidence that this damage contributes to aging and leads to a host of illnesses, including cancer and heart disease.Consuming more antioxidants helps provide the body with tools to neutralize harmful free radicals. It's estimated that there are more than 4,000 compounds in foods that act as antioxidants. The most studied include vitamins C and E, betacarotene and the mineral selenium.

Many people think "supplements" when they think about getting more antioxidants. The supplement aisle, however, is not the only place to find these important compounds. Better places include the produce section, the frozen fruit and vegetable section and the whole grains section of your supermarket. Why? Because the foods in these sections come packaged with other complementary nutrients and phytochemicals. They can provide better insurance than supplements that you're getting the antioxidants you need in the right amount and form.

Here are some good food sources of the four most studied antioxidants.
Beta-carotene -- The most studied of more than 600 different carotenoids that have been discovered, beta-carotene protects dark green, yellow and orange vegetables and fruits from solar radiation damage. It is thought that it plays a similar role in the body. It is found in many foods that are orange in colour including, **Carrots,** squash, broccoli, sweet potatoes, tomatoes, kale, collards, cantaloupe, peaches and apricots. are particularly rich sources of beta-carotene.

Vitamin C -- Also called ascorbic acid, vitamin C is a water-soluble vitamin found in all body fluids, so it may be one of our first lines of defence. This powerful antioxidant cannot be stored by the body, so it's important to get some regularly -- not a difficult task if you eat fruits and vegetables. Important sources include citrus fruits, green peppers, broccoli, green leafy vegetables, strawberries, raw cabbage and potatoes. It is also found in cereals, beef, poultry and fish.

Vitamin E -- A fat-soluble vitamin also known as alpha-tocopherol, it can be stored with fat in the liver and other tissues, vitamin E is promoted for a range of purposes -- from delaying aging to healing sunburn. While it's not a miracle worker, it's another powerful antioxidant. Important sources include wheat germ, nuts (almonds), seeds, whole grains, green leafy vegetables, broccoli, mangos corn and soybean oil and fish-liver oil.

Selenium -- Selenium is a mineral, not an antioxidant nutrient. However, it is a component of antioxidant enzymes. This mineral is thought to help fight cell damage by oxygen-derived compounds and thus may help protect against cancer. It is best to get selenium through foods, as large doses of the supplement form can be toxic. Good food sources include fish, shellfish, red meat, grains, eggs, chicken and garlic. Vegetables can also be a good source if grown in selenium-rich soils.
The amount of selenium in soil, which varies by region, determines the amount of selenium in the foods grown in that soil. Animals that eat grains or plants grown in selenium-rich soil have higher levels of selenium in their muscle. In the United States, meats and bread are common sources of dietary selenium. Brazil nuts also contain large quantities of selenium.

Lutein, best known for its association with healthy eyes, is abundant in green, leafy vegetables such as collard greens, spinach, and kale.

Lycopene is a potent antioxidant found in tomatoes, watermelon, guava, papaya, apricots, pink grapefruit, blood oranges, and other foods. Estimates suggest 85 percent of American dietary intake of lycopene comes from tomatoes and tomato products.

Biology

All living organisms contain complex systems of antioxidant **enzymes** and chemicals, some to combat oxidative damage to cellular components and others to regulate and sustain natural cellular processes such as **oxidative phosphorylation** and the formation of **disulfide bonds.**

Oxidative damage

One major action of antioxidants in cells is to prevent damage due to the action of **reactive oxygen species.** Reactive oxygen species include **hydrogen peroxide** (H_2O_2), **hypochlorous acid** (HOCl) , and **free radicals** such as the **hydroxyl radical** (·OH) and the **superoxide anion** (O_2^-).[3] These molecules are unstable and highly reactive, and can damage cells by chemical chain reactions such as lipid peroxidation, or formation of DNA adducts that can lead to **oncogenic mutations** or cell death if not reversed by **DNA repair** mechanisms. All cells therefore contain antioxidants that serve to reduce or prevent this damage.

ATP generation and cellular maintenance

Antioxidants are especially important in the **mitochondria of eukaryotic** cells, since the use of oxygen as part of the process for generating energy produces reactive oxygen species. The process of aerobic metabolism requires oxygen because it serves as the final resting place for electrons generated by the oxidation steps of the citric acid cycle (i.e. oxygen is the final "electron acceptor" of the redox reactions). However, the superoxide anion is produced as a by-product of this reduction of oxygen in the electron transport chain. Specifically, the reduction of **coenzyme Q** in complex III is a major source of superoxide anion, since a highly reactive free radical is formed as an intermediate ($Q\cdot^-$). This unstable radical can lead to electron "leakage"; instead of moving along the well-controlled reactions of the electron transport chain, the electrons jump directly to molecular oxygen, forming the superoxide anion.[4]

The **redox** state of the cell's interior is tightly regulated. The **cytoplasm** is a reducing environment; however, proteins synthesized for **secretion** often must be oxidized - particularly to form **disulfide bonds** between **cysteine** residues - before export, which normally takes place in the **endoplasmic reticulum** and **Golgi apparatus.**

The **thioredoxin** system contains the 12-k**Da** protein **thioredoxin** and its companion **thioredoxin reductase**. Thioredoxin is present in all sequenced organisms except Tropheryma whipplei (the bacteria that cause **Whipple's disease**).[5] The active site of thioredoxin consists of two **neighboring cysteines**, as part of a highly conserved CXXC **motif**, that can cycle between an active **dithiol** form (reduced) and an oxidized **disulfide** form. In its active state, thioredoxin acts as an efficient reducing agent, scavenging reactive oxygen species and maintaining other proteins in their reduced state. After being oxidized, the active thioredoxin is regenerated by the action of thioredoxin reductase, and thioredoxin reductase is in turn reduced by **NADPH**.[6]

The **glutathione** system includes glutathione, **glutathione reductase,** and **glutathione peroxidase**. Glutathione peroxidase is an enzyme with four **selenium**-containing groups that catalyze the breakdown of hydrogen peroxide and protects lipids in cell walls from peroxidation. There are at least four different glutathione peroxidase genes in animals. Glutathione peroxidase 1 is the most aboudant and is a very efficient scavenger of H2O2, while glutathione peroxidase 4 is mainly a scavenger of lipid hydroperoxides. Glutathione is absolutely nescessary for animal life; mice genetically engineered to be deficient in glutathione biosynthesis die before birth. However, glutathione peroxidase 1 is dispensable for life: mice genetically engineered to lack this enzyme have a normal lifespan.

Superoxide dismutase (SOD) is a class of closely related enzymes that catalyse the breakdown of the highly reactive **superoxide** anion into oxygen and hydrogen peroxide. SOD proteins are present in almost all aerobic cells and in extracellular fluids. Each molecule of superoxide dismutase contains atoms of **copper, zinc, manganese or iron**. SOD that is formed in the **mitochondria** contains manganese (MnSOD). This SOD is synthesized in the matrix of the mitochondria. SOD that is formed in the cytoplasm of the cell contains copper and zinc (CuZnSOD). There also exists a third form of SOD in extracellular fluids, termed EC-SOD (also containg Copper and Zinc at the active sites). MnSOD seems to be the most biologically important of these three since mice lacking this gene die soon after birth. Mice lacking CuZnSOD have a shortned lifespan, while EC-SOD lacking mice have minimal defects.[7]

Catalase is a widely occurring enzyme containing four iron atoms in a 500 amino acid protein. Catalase catalyses the conversion of hydrogen peroxide to water and oxygen at rates of up to 6,000,000 molecules per minute. Catalase has a secondary role oxidising toxins including formaldehyde, formic acid and alcohols. The exact role of catalase in animals is still debated since humans with genetic deficiency of catalase ("acatalesemia") suffer few ill effects and genetic deletion of the catalase in gene in mice is not detrimental either.

Peroxiredoxins catalyze the reduction of hydrogen peroxide, alkyl peroxides, **hydroperoxides** as well as peroxynitrite. There are presently six different peroxiredoxins known. Genetic ablation of peroxiredoxin 1 or 2 causes shortned lifespan and hemolytic anemia in mice.

What we can do?

Every time they neutralise a free radical, the antioxidant loses an electron and stops being able to function as an antioxidant. This is why you must continually re-supply your body with the vitamins and other chemicals that act as antioxidants.

In light of the role free radicals play in the onset of aging and disease, it is important to ensure our diets include a rich, diverse and constant supply of antioxidants. These protective agents can be found abundantly in vegetables, fruits, nuts and seeds and are particularly high in superfoods, like Carrots!

Research is divided over whether or not antioxidant supplements offer the same health benefits as antioxidants in foods. Antioxidants are compounds in foods that scavenge and neutralise free radicals. Evidence suggests that antioxidant supplements don't work as well as the naturally occurring antioxidants in foods such as fruits and vegetables.

The Carrot Museum recommends that people eat a wide variety of fresh fruits, vegetables, whole grains, lean meats and dairy products every day. The diet should include five daily serves of fruit and vegetables. One serve is a medium-sized piece of fruit or a half-cup of cooked vegetables. Of course, in addition to eating plenty of fruits and vegetables, getting regular exercise and abstaining from tobacco use are also critical to a healthy lifestyle But as always - see your doctor or dietitian and health physician for specific advice.

Remember : These informations given here are for informational purposes only and is not intended to be a replacement for medical advice from your personal physician.

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II.APPLICATIONS IN NUTRITION AND MEDICINE

II.Applications in nutrition and medicine

Health preservation

Antioxidants are chemicals that reduce oxidative damage to cells and biomolecules. Researchers have found a high correlation between oxidative damage and the occurrence of disease. For example, **low density lipoprotein** (LDL) oxidation is associated with **cardiovascular disease.** The process leading to **atherogenesis, atherosclerosis,** and cardiovascular disease is complex, involving multiple chemical pathways and networks, but the precursor is LDL oxidation by free radicals, resulting in inflammation and formation of **plaques.**

Research suggests that consumption of antioxidant-rich foods reduces damage to cells and biochemicals from free radicals. This may slow down, prevent, or even reverse certain diseases that result from cellular damage, and perhaps even slow down the natural aging process. This is the basis for the **free-radical theory** of aging.

Some of the reactions in the body that produce free radicals involve metal ions. Some antioxidants, such as the **tannins** in walnuts and tea, **chelate** (wrap around) metal ions. This not only reduces the formation of ion-dependent free radicals, but also prevents the metal ions from oxidizing cells and biochemicals directly.

Some studies suggest that by destroying free radicals and reducing cellular damage, antioxidants in the diet can have positive health effects, such as preventing **macular degeneration** (studied in the **Age-Related Eye Disease Study);**[8] maintaining the **immune system;**[9] potentially preventing **neurodegeneration** due to oxidative stress;[10] preventing **DNA damage;**[11] and lowering the risk of **cardiovascular disease.**[12] Any specific antioxidant may perform only a small fraction of these functions. The mixed results from controlled studies using antioxidant vitamins suggest that other antioxidant substances in fruit and vegetables at least partially explain the better health of those who consume more fruit and vegetables.[13] Dietary antioxidants are not the primary antioxidant inside the body, and there are still many questions as to how **polyphenols** and other dietary antioxidants protect cells and biochemicals from oxidation. Some antioxidants preserve, or even recycle, other antioxidants such as **vitamin E.**

Adverse effects

Relatively strong reducing acids can have anti-nutritional effects by binding to dietary minerals in the gastrointestinal tract and preventing them from being absorbed. Notable examples are **oxalic acid and phytic acid**, which are high in plant-based diets. Some **tannins** also have this negative characteristic. **Calcium** and

iron deficiencies are not uncommon in mideastern diets where there is high consumption of **phytic acid** present in beans and unleavened **whole grain** bread. These anti-nutrients can result in deceptively high **oxygen radical absorbance capacity** (ORAC) ratings given to various "healthy" beverages and foods, particularly:

Foods	**Reducing acid**
Cocoa and chocolate, spinach, and berries	Oxalic acid
Whole grains, maize	Phytic acid
Tea	Tannins

Other extremely powerful **nonpolar** antioxidants such as **eugenol** also happen to have toxicity limits that can easily be exceeded with the misuse of **essential oils.**
While antioxidants supplementation is widely hypothesized to prevent the development of cancer, antioxidants may, paradoxically, interfere with cancer treatments**.**[14] One explanation for this effect is that the growth-promoting environment of cancer cells leads to high levels of redox stress under baseline conditions, and this makes cancer cells more susceptible than normal cells to the further stress of chemotherapy or radiation therapy. So by reducing the redox stress in cancer cells, antioxidant supplements could decrease the effectiveness of the therapy designed to kill them.

Calorie restriction
Virtually all studies of mammals have concluded that a **restricted calorie diet** (CR) extends median and maximum lifespan (CR is almost the only protocol to have achieved this). This benefit appears to be at least partly due to substantially reduced oxidative stress**.**[15] As food produces free radicals (oxidants) when metabolized, antioxidant-rich diets are thought to stave off the effects of aging significantly better than diets lacking in antioxidants.

Physical exercise
During exercise, oxygen consumption can temporarily increase by a factor of more than 10.[16] This leads to a temporary large increase in the production of oxygen free radicals, resulting in increased cell damage contributing to muscular fatigue during and after exercise. The body uses antioxidants to reduce the amount of such damage. The inflammatory response that occurs after strenuous exercise is also associated with increased occurrence of free radicals, especially during the 24 hours after an exercise session. In this phase too, antioxidants in the body reduce the damage. The immune system response to damage done by exercise peaks 2 to 7 days after exercise, the period during which adaptation resulting in greater fitness is greatest. During this process, free radicals are used by **neutrophils** in the immune system to identify damaged tissue. As a result, excessive antioxidant levels have the potential to inhibit recovery and adaptation mechanisms.[17]

There is a popular view that those who undertake vigorous exercise can benefit from increased consumption of antioxidants, but an examination of the literature finds support that this is the case only for certain antioxidants at certain levels, and some evidence that very large intake of some antioxidants may be detrimental to recovery from exercise. There is strong evidence that one of the adaptations that result from exercise is a strengthening of the body's antioxidant defenses, particularly the glutathione system, to deal with the increased oxidative stress.[18] It is possible that this effect may be to some extent protective against diseases which are associated with oxidative stress, which would provide a partial explanation for the lower incidence of major diseases and better health of those who undertake regular exercise.
The antioxidant system that protects **lipid membranes** from free radicals includes vitamin E, beta-carotene, vitamin A, and coenzyme Q10. The system that scavenges free radicals in the water based **cytoplasm** includes vitamin C, glutathione peroxidase, superoxide dismutase, and catalase. The effect of each of the exogenous antioxidants needs to be examined separately, although they work in a co-operative manner.

The body of research suggests no benefits from supplementing with vitamin A above normally recommended levels. Recent well-designed studies suggest there are no ergogenic benefits from vitamin E (except for those who do exercise at high altitude)[19-21] despite its key role in preventing lipid membrane peroxidation. For example, 6 weeks of vitamin E supplementation had no effect on muscle damage indicators in ultramarathon runners.[22] Although selenium is essential to the glutathione antioxidant system which, as mentioned above, is upregulated by exercise, there is no evidence that supplementation with selenium above the **RDA** is of any ergogenic benefit. However, for vitamin C there is considerable evidence that vitamin C requirements are greater in those who do vigorous exercise, with plasma levels falling with intake of 100mg (well over the accepted RDA) and around 300mg per day being required to maintain blood plasma levels.[23] There is some evidence that supplementation with vitamin C increased the amount of intense exercise that can be done, and lowered the heart rate while doing it (which is indicative of greater efficiency),[24] and that vitamin C supplementation before strenuous exercise reduces the amount of muscle damage.[25]

However, some other studies found no such effects, and some research suggests that supplementation with amounts as high as 1000 mg inhibits recovery,[26] although the very short pre-exercise supplementation period in this study may have influenced the results. There is strong evidence that vitamin C supplementation reduces upper respiratory tract infections in ultra-endurance athletes.[27]
In summary, a diet with at least 300 mg of vitamin C is of benefit to those who undertake high intensity or high volume exercise, but it is not clear that normal requirements for vitamin A, vitamin E or selenium are increased.

Clinical trials

Although some levels of antioxidant vitamins and minerals in the diet are required for good health, there is considerable doubt as to whether antioxidant supplementation is beneficial, and if so, which and what amount of antioxidant(s) are optimal.One study of lung cancer patients found that those given beta-carotene supplements had worse prognoses. Two 1994 studies found an increased rate of lung cancer in smokers supplementing with beta carotene. This is believed to be due to antioxidant interference with the body's normal use of localised free radicals e.g. **nitric oxide** for cell signalling. Due to the complex nature of the interactions of antioxidants with the body, it is difficult to interpret the results of many experiments. In vitro testing (outside the body) has shown many natural antioxidants, in specific concentration, can halt the growth of or even kill cancerous cells.

In the early 1990s, it was hypothesized that oxidation of LDL **cholesterol** contributes to heart disease, and several observational studies found that people taking Vitamin E supplements had a lower risk of developing heart disease..[28] Taken together, this led researchers to conduct at least seven large clinical trials testing the effects of antioxidant supplement with Vitamin E, in doses ranging from 50 to 600 mg per day. However, none of these trials found a statistically significant effect of Vitamin E on overall number of deaths or on deaths due to heart disease.[29]

While several trials have investigated supplements with high doses of antioxidants, the "*Supplémentation en Vitamines et Mineraux Antioxydants*" (SU.VI.MAX) study tested the effect of supplementation with doses comparable to those in a healthy diet.[30] Over 12,500 French men and women took either low-dose antioxidants (120 mg of ascorbic acid, 30 mg of vitamin E, 6 mg of beta carotene, 100 µg of selenium, and 20 mg of zinc) or **placebo** pills for an average of 7.5 years. The investigators found there was no statistically significant effect of the antioxidants on overall survival, cancer, or heart disease. However, a subgroup analysis showed a 31% reduction in the risk of cancer in men, but not women. The authors interpreted these results as suggesting that "an adequate and well-balanced supplementation of antioxidant nutrients, at doses that might be reached with a healthy diet that includes a high consumption of fruits and vegetables, had protective effects against cancer in men."

Measurement

Oxygen radical absorbance capacity (ORAC) has become the current industry standard for assessing antioxidant strength of whole foods, juices and food additives.[31] Other measurement tests include reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating.[32]

Food preservatives

Antioxidants used as **food additives to help guard against food deterioration** include:

- Ascorbic acid **(vitamin C)**
- **Tocopherol**-derived compounds
- **BHA, BHT, EDTA**
- **Tert-Butylhydroquinone**
- **Citric acid**
- **Acetic acid** - found in vinegar; used for pickling
- **Pectin**
- **Rosmarinic acid** - in the form of the herb rosemary and Italian seasoning mixtures in naturally or minimally processed foods, and pet foods.

Food Sources

See also List of antioxidants in food

Antioxidants are found naturally in varying amounts, in vegetables, fruits, grain cereals, legumes, nuts etc. Antioxidant sources [33] include:

- Fruits: **berries**
- Vegetables: peppers and **spinach.**
- Fungi: mushrooms
- Whole grain cereals: barley, millet and maize.
- Nuts: pecans, pistachios, and almonds [34]
- Cocoa products: **chocolate.**
- Drinks: **Coffee, wines, and teas .**

Dietary supplements

See also List of phytochemicals and foods in which they are prominent

Since the discovery of vitamins, it has been recognized that antioxidants from the diet are essential for healthful lives in humans and many other mammals. More recently, a large body of evidence has accumulated that suggests supplementation of the diet with various kinds of antioxidants can improve health and extend life. Many **nutraceutical** and health food companies now sell formulations of antioxidants as dietary supplement. These supplements may include specific antioxidant chemicals, like **resveratrol** (from grape seeds), combinations of antioxidants, like the "ACES" products that contain beta carotene (provitamin **A**), vitamin **C**, vitamin **E** and **S**elenium, or specialty herbs that are known to contain antioxidants such as **green tea and jiaogulan.**

In fuels

Some antioxidants are added to liquid industrial chemicals, most often **fuels** and **lubricants** to prevent oxidation, and in gasolines to prevent polymerization leading to **gumming.** Some examples are:

- **AO-22 (N,N'-di-2-butyl-1,4-phenylenediamine),** for turbine oils, transformer oils, hydraulic fluids, waxes, and greases
- **AO-24 (mostly N,N'-di-2-butyl-1,4-phenylenediamine),** blended for low-temperature handling)
- **AO-29 (2,6-di-tert-butyl-4-methylphenol**), for turbine oils, transformer oils, hydraulic fluids, waxes, greases, and **gasolines**
- **AO-30** (alkylated phenols, mostly **2,4-dimethyl-6-tert-butylphenol** (>97%)), for **jet fuels** and gasolines, including aviation gasolines
- **AO-31** (alkylated phenols, mostly **2,4-dimethyl-6-tert-butylphenol** (>72%)), for jet fuels and gasolines, including aviation gasolines
- **AO-32** (alkylated phenols, mostly **2,4-dimethyl-6-tert-butylphenol** (>55%), and **2,6-di-tert-butyl-4-methylphenol** (>15%)), for jet fuels and gasolines, including aviation gasolines
- **AO-36** (alkylated phenols), for gasolines
- **AO-37** (alkylated phenols, mostly **2,6-di-tert-butylphenol),** for jet fuels and gasolines, widely approved for **aviation fuels**

Antioxidants are frequently used together **with metal deactivators and corrosion inhibitors.**

Researches about antioxidants have expanded and diversified within this last decade. Over the past five years, more researches have been published on vitamin C, E and on Beta Carotene. These vitamins, along with other minerals such as Selenium, Copper, Zinc, or Manganese have been strongly linked to the prevention (and possible treatments for) of numerous diseases from heart diseases and cancers to cataracts and arthritis as well as to the regulation of the immune system and the prevention of premature aging. An antioxidant is any compound that fights the destructive effects of free radicals oxidants. This antioxidant system is composed of enzymes, vitamins, minerals, and other substances produced in the body or obtained through the diet. Antioxidants act as scavengers and prevent the formation of free radicals. They could also bind to and neutralize these reactive substances before they can actually damage the tissues.

Some Oxygen Scavengers

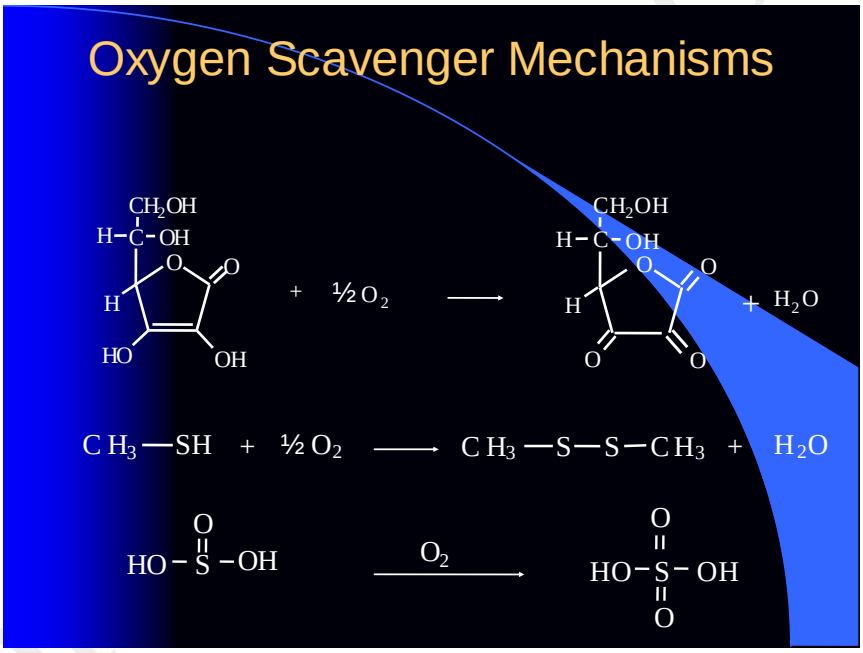
- Ascorbic acid
- Ascorbic palmitate
- Erythorbic acid
- Sulfites

ANTIOXIDANT AND FREE RADICALS

It is no secret that antioxidants are "good" and free radicals are "bad". But most of us have no idea what they mean. Antioxidants neutralized harmful chemicals called "free radicals". This process occurs in the body on a constant basis.

What are Free-Radicals?

Free radicals are extremely unstable substances, which in the presence of oxygen will turn any shiny metal object into an ugly rusted object very quickly. A normal oxygen atom in the human body contains four pairs of electrons. During the course of normal metabolism, a single electron may be lost. The result is "free radical" an unstable molecule which has a strong drive to replace its missing electron, taking one from a neighboring molecule, causing cell structure damage resulting in degenerative disease , premature aging, etc. For example, H2O or water, will typically not react with DNA, the master molecule guiding every cell on the planet. But the same H2O molecule, hit by little ultraviolet radiation- perhaps from sunlight, converts to HO- free radical. This OH-free radical would be very happy to mess with DNA molecule- such occurrences are very common in the human body . Most cell are well equipped with the molecular machinery necessary to repair the damage DNA and eliminate the free radicals damage that goes unrepaired, resulting in a mutant cell, it takes seed to malignant tumor.



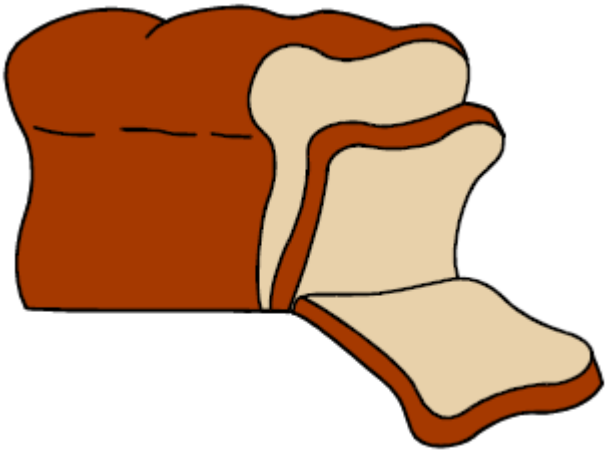
Free radicals damage has been implicated in the pathogenesis of many diseases. For example, it is believed that free radicals can react with low density lipoproteins (LDLs), the "bad cholesterol" in the blood. Damaged LDLs can adhere to the inner well of blood vessels forming plaques that clot arteries and cause heart attacks and strokes.

Types of free radicals damages:

1. **Damage to fat compounds:** The fatty membranes surrounding the cells being the prime target to free radicals attacks. The damaged membranes then loose its ability to transport oxygen, nutrients or water to the cells.

2. **Damage to protein molecules:** Free radicals also attack the nucleic acid which comprise the genetic code within each cell. The nucleic acids function is to regulate the normal cell function, growth and also to repair the damaged tissues.
3. **Cell damage:** Damages done to the chromosoins and nucleic acids might initiate the growth of abnormal cells, which is the first step in cancer development.
4. **Lysosomes damages:** Lysosomes are little sacs in the cell that contain degenerative enzymes. The enzymes leak out when the membrane cell breaks and they start digesting the cell itself, spreading to nearby cell causing a chain reaction of destruction which, eventually, will lower the immune system resistance.

Free radicals arise from fatty food, smoking, alcohol, environmental pollutants, hydrogen peroxide, pollutants, ozone, toxins, carcinogen toxins etc. The vast majority of free radicals come from within the body, an unavoidable byproduct of living system.



Under normal conditions, the body handles free radicals formed by the breakdown of compounds through the process of metabolism. However, the ability of the body to neutralize free radicals is generally becoming more difficult because of an increased exposure to pollutants.
Once again, Mother Nature holds the solution. Most of the colorful fruits and vegetables are endowed with molecules possessing an exceptional ability to protect the cell so that the free radicals be left with no chance to actually cause any damages. These "cell-savers" are called "Antioxidants."

Antioxidants get their name because they combat oxidation. Oxidation is a reaction in which a molecule looses an electron. The two major sources of antioxidants are:

1. Those that you get from food or food supplements
2. Those produced within your own body (for example, Glutathione peroxidase breaks down Glutethione into non-toxic products)

Some antioxidants, like the Flavonoids, found in the skin and seeds of fruits, possess the ability to physically capture free radicals until these are actually removed from the body. Others, like Sulphorophane, found in broccoli, tend to enhance the body’s own free radical scavenging mechanism. And finally, the ones like L. Limonene, phytochemical found in citrus fruit peels, can actually perform both actions.

Some popular antioxidants today include **Vitamin E, Vitamin C, Vitamin A**, which can be taken under a health supplement form or through **fruits, vegetables, fish oil, Ginkgo Biloba, green tea, sesame oil, and Genistein from soy bean** shown to be cancer preventive. Some antioxidants come from minerals, such as **selenium, Zinc ..** etc. The list of dietary antioxidants goes on and on, and scientist are continually discovering more.

Singlet Oxygen Quenching of Tocopherols

$^{1}O2 + {}^{1}\text{Tocopherol} \longrightarrow {}^{3}O2 + {}^{3}\text{Tocopherol}$

$^{3}\text{Tocopherol} \longrightarrow {}^{1}\text{Tocopherol}$

Many nutrients such as selenium, copper, zinc...etc... are considered antioxidants because they work together in conjunction with an antioxidant enzyme and are necessary for the enzyme to function properly. For instance, selenium is essential for the production of the glutathione peroxidase enzyme which protects the red cells and cell membranes from free radicals damages. Other nutrients, such as the vitamin C, E, or beta carotene, function as antioxidants independent from an enzyme. Vitamin C is water-soluble, thus, it reduces the free radicals damages caused within the watery area of the body such as the blood stream, lymphatic fluid and the fluid between and within the cells. The beta carotene also protects the fatty parts of the cell membranes. Individual antioxidants works as a team to defend the body against free radicals. For instance, selenium and vitamin E assist each other in protecting the cell membranes from free radicals’ attacks; And Vitamin C can restore Vitamin E should the latest be damaged.
The body’s need for antioxidants nutrients might vary from individual to individual based on existing diseases and on the degree of exposure to free radicals (e.i. pollution, radiation, smoke, poor diet, inflammatory diseases...).

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III. ANTIOXIDANTS AND IMMUNITY

III. Antioxidants and Immunity

The immune system is the body's defense against the invasion of foreign substances such as microorganisms. It is a complex network of specialized tissues, organs, cells and chemicals whose primary purpose is to recognize and destroy the foreign invader. The lymph nodes and vessels, spleen, bone marrow, and thymus gland are examples of organs and tissues participating in the immune system. The cells and secretions of the immune system include specialized white blood cells called "T-Lymphocytes" and "B- Lymphocytes", a chemical called "Interferon", produced by the T- Lymphocytes, monocytes, macrophages, as well as the antibodies produced by the T- Lymphocytes.

A well-functioning immune system recognizes any unwanted microorganisms, abnormal and potentially cancerous cells, or any foreign substances in the body and then destroys them. However, should our immune system be malfunctioning, our body's defense would end up weakened, which in turn may lead to the development of various disorders. Poor nutrition is one of the most frequent reason causing the immune system not to perform at its best. How severely the immune system is damaged depends on which vitamin or mineral is deficient and to which extent it is missing in the diet. Every immune system functions are affected during malnutrition. In contrast, an optimal intake of vitamins and minerals along with the consumption of low fat food enhances the immune system and reduces the risks of developing diseases and infections.

Immunity and Antioxidant Nutrients:

The antioxidant nutrients - Vitamin E, Beta Carotene, and Vitamin C, are important in maintaining an optimal immune system condition. These vitamins prevent the cells and tissues from being damaged by free radicals.

- **Vitamin A** stimulates the immune system and decreases the risk of infections. A research review at the University of Queensland Medical School in Australia, showed that an adequate intake of vitamin A in children reduces the death rate by one third, leading to a two percent decrease in death causes by respiratory diseases.

Agric. Food Chem. **44**, 701-705.

- **Beta Carotene** enhances our immunity by increasing T-Lymphocytes and B-Lymphocytes activities, protecting macrophages, and facilitating the communication between the immune system cells. Beta Carotene also lessens the immune system damages associated with the exposure to ultraviolet radiation under sunlight or X-ray.

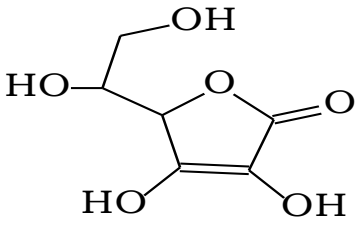
  Physical Quenching Mechanism of β- Carotene on Singlet Oxygen

  $^1O_2 + {}^1\beta\text{-Carotene} \xrightarrow{\text{Energy Transfer}} {}^3O_2 + {}^3\beta\text{-Carotene}$

  $^3\beta\text{-Carotene} \xrightarrow{\text{Radiationless Transfer}} {}^1\beta\text{-Carotene}$

- **Vitamin C** strengthens the immune system by increasing the production of T and B-Lymphocytes, and other white blood cells. This antioxidant also increases the white blood cells ability to destroy microorganisms and increases the production of interferon.

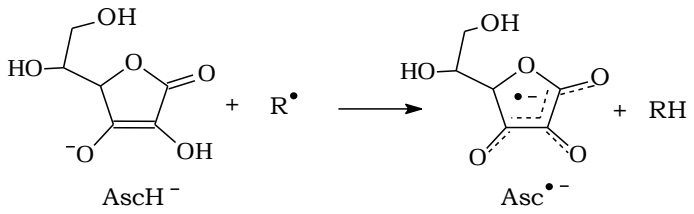
Ascorbic Acid Structure



($AscH_2$)

Ascorbate Chemistry Society For Free Radical Biology and Medicine Buettner & Schafer 2

$AscH^-$ is a Donor Antioxidant



$AscH^-$ donates a hydrogen atom ($H^\bullet$ or $H^+ + e^-$) to an oxidizing radical to produce the resonance-stabilized tricarbonyl ascorbate free radical. $AscH^\bullet$ has a pK_a of -0.86; thus, it is not protonated in biology and will be present as $Asc^{\bullet -}$.

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Vitamin E is an essential component of all cell membranes including the outer cell membranes as well as the membranes surrounding cell's nucleus. (where the genetic code is stored), and the mitochondria (the cell's power center). Vitamin E and the other antioxidants prevent damage caused by free radicals and help maintain the normal structure and functions of the immune cells

- and tissues. Vitamin E improves the T-Lymphocyte activity. If applied directly onto the skin, vitamin E can prevent the immune suppression caused by as little as 30% exposure to UV light.

A typical American or Chinese diet is often too low in antioxidants. Therefore suggesting an intake of supplements might be necessary.

FOOD	ANTIOXIDANT ACTIVITY (TE/100 Grams)
Red Grapes	1350
Red Cabbage	1000
Broccoli Flowers	500
Spinach	500
Green Grapes	400
Tomato	300
Green Beans	175
Green Cabbage	150
Lima Beans	1055
Red Beans	11459
Blueberries	3300
Raisins	5900
Wheat Bran	4620
Wheat Flour (refined)	600

Immunity and other Vitamins and Minerals:

Several other vitamins are also essential for the optimal performance of the immune system.

A deficiency in vitamin B6 can reduce the lymphocyte number, the antibodies' production, and might contribute to an immune suppression associated with aging and HIV infection. Inadequate vitamin D intake impairs the immunity system while a vitamin D-rich diet will correct the impairment.

Zinc is essential for the antibodies production , the normal thymus gland function, and the effectiveness of specialized lymphocytes called "helper cells". An optimal intake of zinc restores the compromised immune functions and reduces the susceptibility of infections. A deficiency in copper might impair the immunity and

increase the risk of infections, especially in premature infants. An adequate intake of selenium stimulates the release of lymphocytes , increases the "natural killer cell" activity, and protects the body from toxic metals that suppress the immune system.

Immunity and Herbs:

There are many reasons for the increased interest in Herbs. Some of these have to do with the increasing incidence of immune deficiency disorders such as AIDS and Chronic Fatigue Syndrome (CFS). It has been reported that certain substances contained in herbs are actually immune enhancing as well as antiviral.

Herbs may contain powerful pharmacologic agents and this fact should be no surprise to anyone. Many of the most important drugs on the market today, are derived from herbs.: for instance, aspirin is derived from the White Willow bark, Colchicine from Meedow saffron, Quinine and Quinidine from Cinchona, Vinblastine and Viucristine (two important anti-cancer drugs) from the Periwinkle. These are only a few examples.

"What is an herb?"

It is a plant or plant part valued for its medicinal, aromatic, or savory qualities. Those prized for their savory or aromatic qualities are culinary herbs or spices as opposed to the medicinal herbs, valued not only for their ability to treat illness but also for their capacity to actually prevent illness. Certain plant-food such as the cruciferous vegetables (cabbage, broccoli, cauliflower...), high fiber-food , carrots, garlic, green leafy vegetables, onions..., can protect us against certain forms of cancers and heart diseases.

- **Ginkgo Biloba:**

The Ginkgo Biloba leaves contain, among others, bioflavonoids, flavoglycosides, proanthrocyanidins (a substance closely related to the bioflavonoids) and a collection of unusual polycyclic structures called "ginkgosids" which are, chemically speaking, polyacetones. These substances produce their therapeutic action by acting as antioxidants, and by inhibiting platelet aggregation.

- **Ginseng:**

Soviet researches have been particularly keen on ginseng, and more particularly the Siberian ginseng, called Eleutherococcus Senticosus. The results of their researches showed that this particular herb possesses the abilities to boost the immunity, inhibit cancer, increase energy and physical stamina. It has also been said to have variable effects on both the blood pressure and blood sugar. Russians cosmonauts have been directed to chew the herbs to fend off infections.

- **Licorice:**

Real licorice comes from the dried unpeeled roots of the Gycyrriltiza glabra plant. Licorice has been used in China for over 3000 years for the treatment of peptic ulcers, sore throat, coughs. It is one of the major Chinese tonic and is well known to revigorate the functions of the heart and spleen. Some of the substances isolated from licorice are of enormous Medical interest. The gyccyrrhizin and glycyrrhetinic

acids have been reported to inhibit the melanoma cell (causing skin cancer in vitro). The glycyrrhetinic acid has inhibited the activity of two powerful tumor promoters in mice.

- **Wild Yam:**

The Mexican wild yam or Barbasco, occupies an important place in the Medical history. In 1940, Russel Marker was the first one to isolate the diosgenin from the bark root of a Dioscorea, thanks to which, progesterone and testosterone could be synthesized. Later, it was discovered that the Mexican wild yam had an even higher amount of diosgenin than previously thought and consequently, the yam became the source and ingredient of the original birth control pill. The Mexican wild yam also contains dihydroepandrosterone, or DHEA, which possesses a number of various beneficial effects on the immune system and the ability to prevent a number of degenerative and infectious diseases. DHEA stimulates T and B-Lymphocytes as well as macrophages by interfering with glucocorticoid immuno-suppression. It protects against both bacterial and viral infections. Researches in areas such as lupus, multiple sclerosis, chronic fatigue, and AIDS show great promises. In general, most people taking DHEA report feelings of increased tolerance to stress, fatigue and illness.

- **Schizandra:**

Schizandra is a Chinese herb regarded as miraculous when it comes to its medicinal properties. It has been used as an astringent for the treatment dry cough, asthma, night sweat, nocturnal seminal emission, and chronic diarrhea. Substances have been isolated from this herb and those appeared to possess some protective actions against liver toxins in mice. Immunomodulating substances have also been isolated and have been shown to possess some cortisone-like effects.

- **Astragalus:**

Astragalus, derived from the root of the Astragalus Membraneceus, is used in nearly half of all Chinese prescriptions. It has a reputation for being a potent energizer and immune stimulant . Chinese researchers have even reported its successful use on cancer patients, in offsetting some of the immune suppressing effects of many western cancer drugs and radiation. Researchers in the US have confirmed some of their findings . Investigators at the University of Texas have reported some strong immune restorative effects in test-tubes studies of cancer cells treated with Astragalus extracts. Astragalus contains a number of potentially therapeutic substances including a polysaccharide called "Astragalus B", which, after having been tested, has been reported to protect against some toxins and against a number of bacterial infections, as well as to stimulate the components of the immune system.

- **Cat Claw:**

Cat Claw or Uncaria Tomentosa, is an herb that grows wild in the highland of the Peruvian Amazon. It has been used for hundreds, perhaps thousands of years by the native Indians for the treatment of a wide range of health problems associated with the immune and digestive systems. Six oxindole alkaloids were isolated from the root of Uncaria Tomentosa. The most immunologically active alkaloid is called

Isopteropodine, and has been shown, in laboratory testing, to have a pronounced enhancement effect on phagocytosis ("phagocytosis"being the ability of the white blood cells and macrophages to attack, engulf, and digest harmful microorganisms or foreign invaders).

- **Grape Seeds:**

Pro-anthocyanidins are one of the greatest recent advance for anti-aging and disease. They are found in vegetables, grape seeds extracts. Pro-anthocyanidins are actually a special class of bio-flavoroids that are powerful antioxidants and free radical scavengers. One of the theories of aging is that, with the production of excess free radicals, the joints, skin and other organs are damaged and the bad cholesterol is oxidized and made more dangerous. As a result of this damages, people age more rapidly and acquire age-related diseases such as arthritis, and cataracts. Pro-anthocyanidins are said to be a more effective antioxidant than vitamin C or E. Researchers at Negasaki University have demonstrated that pro-anthocyanidins destroy free rdicals 50 times more effectively than vitamin C or E.

The immune system can always be strengthened or stimulated to an optimal level. Our diet is only one of the several factors that can either improve or suppress our immunity. Suggesting food supplements may be very helpful.Moderate, regular exercises decreases the risk of infections, illness, and might prevent age decline in immune functions. A generally healthy lifestyle also improves and reinforces our immune system.

Antioxidants and Eye Disorders:
Antioxidants, fish oil, and several vitamin deficiencies might alter our vision and the health of our eyes. Recent researches suggest that many eyes disorders may not be an inevitable effect of aging and thus, might be preventable with an adequate intake of antioxidants.

The lens of the eye filters the ultraviolet light, a potent source of highly reactive compounds (free radical) and is thereby exposed to higher levels of these reactive substances . Free radicals damage the protein within the lens and might form protein "clumps" that scatter light and contribute to the formation cataract. A diet rich in antioxidant nutrients protects against the formation and progression of cataract by counteracting the damaging free radicals. A high intake of antioxidants supplements also reduces the risks of macular degeneration, a common vision impairment in the elderly.

The major antioxidants in the lens of the eye are vitamin C and glutathione. When cataracts form, the level of glulathione in the eye decreases. Vitamin C is needed to activate the glutathione. An highest concentration of vitamin C is found in the aqueous humor (where the level of vitamin C can be 20 times the amount found in blood). The retina contains the lowest concentration of vitamin C in the eye tissues. The major function of the vitamin C is to act as a protector against oxidative damage, particularly the one involving light induced damages. High levels of vitamin C are linked to a low cataract risk.

Glulathione reductase is an enzyme which protects the antioxidants glulathione, vitamin B2 ,Riboflavin, is needed to activate this enzyme. So eventhough we do not usually think of vitamin B2 as being an antioxidant, it does lead to antioxidant activity in the eye. Some of the symptoms associated with a deficiency in vitamin B2 are as follow: damage to the eye tissue, poor vision, burning, itching, and increased blood vessel in the eye. Selenium does active the glutathione peroxidase which in turn helps glutathione act as an antioxidant. The Nurses Health studies reported that people eating the highest dietary level of beta carotene had a 27% lower risk of forming cataract than those consuming a lesser amount.

The antioxidant beta carotene lessens the ultraviolet light damage to the retina and scavenges the free radicals that might damage the eye. Beta carotene converts to vitamin A in the body and is often called the "safest form" of vitamin A. Besides its higher level of safety, beta carotene is different from vitamin A in another way: It is a much stronger antioxidant. Vitamin A binds with a special protein in the eye that makes vision possible in dim light. Night blindness or poor dark adaptation, is an early sign of poor vitamin A intake.

Other antioxidant possibilities: Lutein and Zeaxanthin (which is similar to beta carotene), two important antioxidants found in spinach. In the Nurses Health Studies, eating spinach, as opposed to any other vegetables is correlated with better cataract prevention. Increasing evidence has linked these two "new" antioxidants to the prevention of macular degeneration, the leading cause of irreversible blindness in elderly.

Catalase and superoxide dismutase (SOD) are enzymes with antioxidant activity within the lens of the eye. Both are dependent upon Zinc. High levels of zinc are found in the retinal pigment epithelium (RPE), a colored layer of cells in the retina. The RPE has an increased exposure to free radicals, while the zinc acts as an antioxidant. An increased intake of zinc or zinc-rich food (such as poultry, whole grain bread...) might delay or prevent macular degeneration and improve visual keenness.

Bilberry is a well-known remedy for poor vision. Researchers studying eye health first became interested in this herb when the bilberry's extract first started showing some positive results in improving both night vision and visual adaptation. As a matter of fact, the bilberry jam was first given as a supplement to the Royal Air Force pilots who were flying nighttime mission during World War II. The bilberry works by accelerating the regeneration of the retinal purple, a substance required for good eyesight.

In combination with the vitamin E, the bilberry halted the progression of senile cataracts in one study (Ann Optalmo Clin. Ocul. 1989; pp.109-115). The result was not a surprise since bilberry contains an substance - bioflovanoid, an natural antioxidant which actually protect vitamin C. The unique blue/violet bilberry bioflovanoid, called "anthocyanosides" is a potent antioxidant preventing from free radicals damages.

Antioxidants and Smoker's Detox

Many of the toxic elements in cigarette smoke are oxidant generators of toxic oxygen form that can cause molecular, cellular and tissue damage. Cigarette smoke contains high amounts of nitrogen oxides, which are themselves free radicals. Nitrogen oxides can react with polyunsaturated fatty acids found in cellular membranes, causing rancidification, rigidifcation and death of cells. vitamin C, Vitamin E in particular, protect against these reactions.

The cancer causing potential of cigarette smoking is well established. Lung cancer associated with smoking accounts for more cancer deaths than any other forms of malignancy. Again, it is the free radical activity that is believed to be the basic carcinogenic factor in smoking. Smoking also causes emphysema and increases the risk of heart attacks, strokes and clots in the legs. And it makes peptic ulcers slower to heal. Non-smokers can also be affected by the noxious agents in cigarette smoke. An large number of people are sensitive to cigarette smoke and have problems breathing in its presence.

Cigarette smoke affects the smoker's and non-smoker's nutritional status. Inhalation of cigarette smoke depletes the tissue of vitamin C and increases the daily need for this water soluble vitamin. Although the RDA for vitamin C is raised to 100mg for smokers, some evidence shows that smokers might require twice that level to

maintain their vitamin C at a similar level than that of non-smokers who only consume 60 mg of that same vitamin. In addition to this fact, smokers are more likely to eat diets low in vitamin C and in other antioxidants nutrients. Vitamin A and beta carotene levels in the lungs and mouth tissue that contain adequate amount of vitamin A are less likely to become cancerous. High doses of vitamin A supplementation reduce the damage to the lungs tissues by air pollution such as cigarette smoke.

Smokers have lower zinc level while zinc supplements might reduce the risk of atherosclerosis in smokers. Women who smoke during pregnancy have low levels of zinc in their blood and are more likely to give birth to zinc deficient babies. These babies are at an increased risk for birth defects and diseases. Levels of folic acid are also low in smokers. Vitamin B6 metabolism is altered by cigarette smoking and the residual effects might last as long as 2 years after cessation of smoking.

- Non-smokers are passive smokers. They inhale both the mainstream smoke (the exhaled cigarette smoke from the smokers) and sidestream (the smoke from the end of the burning cigarette) . Sidestream contains greater amounts of dangerous gases than the smoke filtered through the cigarette before entering the smoker's lungs. It contains twice as much tar and nicotine, 3 times more benzopyrene (a cancer causing substance), 3 times more poisonous carbon monoxide, and 73 times more ammonia. The inhalation of carbon monoxoide reduces the oxygen supply to the heart, brain, and other tissues. And the risk for developing lung cancer, and respiratory diseases increases in both smokers and non-smokers.

Antioxidants are clearly the first line of defense for those exposed to smoke pollutants.

ANTIOXIDANT ACTIVITY*

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a

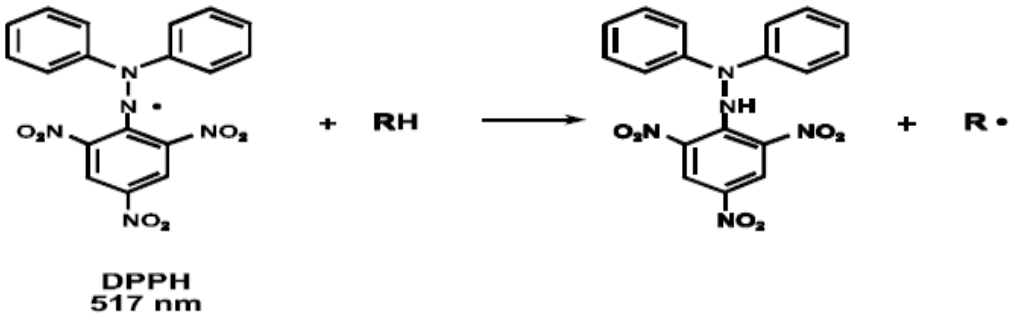
wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in foods have been substantially investigated and reported in the literature by Miller and Rigelhof et.al (1,2).

METHOD CONSIDERATIONS

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. In recent years, oxygen radical absorbance capacity assays and enhanced chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum and other biological fluids. These methods need special equipment and technical skills for the analysis. These types of methods published in the literature for the determinations of antioxidant activity of foods involve electron spin resonance (ESR) and chemiluminescence methods. These analytical methods measure the radical-scavenging activity of antioxidants against free radicals like the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, the superoxide anion radical (O2), the hydroxyl radical (OH), or the peroxyl radical (ROO). The various methods used to measure antioxidant activity of food products can give varying results depending on the specificity of the free radical being used as a reactant. There are other methods which determine the resistance of lipid or lipid emulsions to oxidation in the presence of the antioxidant being tested. The malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) (1) assays have been used extensively since the 1950’s to estimate the peroxidation of lipids in membrane and biological systems.

These methods can be time consuming because they depend on the oxidation of a substrate which is influenced by temperature, pressure, matrix etc. and may not be practical when large numbers of samples are involved. Antioxidant activity methods using free radicals are fast, easy and simple. The ABTS [2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation (2) has been used to screen the relative radical-scavenging abilities of flavonoids and phenolics through their properties as electron- or H-donating agents. Prior et al. (3) have used the Oxygen Radical Absorbance Capacity (ORAC) procedure to determine antioxidant capacities of fruits and vegetables. In the ORAC method, a sample is added to the peroxyl radical generator, 2,2’- azobis(2-amidinopropane)dihydrochloride (AAPH) and inhibition of the free radical action is measured (4) using the fluorescent compound, Bphycoerythrin or R-phycoerythrin. Phenolic and polyphenolic compounds

constitute the main class of natural antioxidants present in plants, foods, and beverages and are usually quantified employing Folin's reagent. Vinson et al. (5) have measured phenolics in fruits and vegetables colorimetrically using the Folin-Ciocalteu reagent and determined the fruit and vegetable's antioxidant capacity by inhibition of low density lipoprotein oxidation mediated by cupric ions.



DPPH
517 nm

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2-Diphenyl-1- picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. It has also been used to quantify antioxidants in complex biological systems in recent years. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity will helps us understand the functional properties of food. Antioxidant activity has been expressed in various ways including the percentage of the reagent used, the oxidation inhibition rate and so on. An easier way to present antioxidant activity of foods would be to reference a common reference standard. One common reference standard, (S)-(-)-6-hydroxy-2,5,7,8-tetramethylchroman-2- carboxylic acid, also known as Trolox, serves as such a common reference standard.

THE DPPH METHOD

A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The structure of DPPH and its reduction by an antioxidant are shown above. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured.

STANDARD ANTIOXIDANTS	ANTIOXIDANT ACTIVITY (TE/100 Grams)
Ascorbate	442,000
Trolox	400,000
Vitamin E	201, 000
BHT	395, 000

Antioxidant compounds may be water-soluble lipid-soluble, insoluble, or bound to cell walls. Hence, extraction efficiency is an important factor in quantification of antioxidant activity of foods. Trolox (as the reference standard) and the samples are reacted with DPPH solution in methanol/water for four hours at 35°C in a vessel mounted on a rotary shaker and the absorbance changes are measured at 517 nm. The quantity of sample necessary to react with one half of the DPPH is expressed in terms of the relative amount of Trolox reacted. Antioxidant activity of a sample is expressed in terms of micromole equivalents of Trolox (TE) per 100 grams of sample, or simply Trolox units per 100 gm or TE/100g.

Trolox units per 100 gm or TE/100g.

RESULTS AND DISCUSSION

The reaction of DPPH with numerous antioxidants has been published and the stoichiometry characterized (6,7). As mentioned above, antioxidants in food may be water soluble, fat soluble, insoluble, or bound to cell walls and thus not necessarily freely available to react with DPPH, hence they react at different rates i.e. differing kinetics, and the reaction will often not go to completion in a reasonable assay time. Therefore, the sample size that can lower the initial absorbance of DPPH solution by 50% has been chosen as the endpoint for measuring the antioxidant activity. This

change is compared to the change induced by Trolox, the reference standard, and the antioxidant activity of the sample is expressed in micromoles of Trolox equivalents (TE) per 100 gm of sample or Trolox units per 100 gm. A difference between this method and other published methods is carrying out the reaction of the sample itself with DPPH in methanol/water. Reacting an aqueous-methanolic DPPH solution with the sample for 4 hours at 35 °C facilitates the extraction of antioxidant compounds from the sample thereby increasing the measured antioxidant activity of the sample. Determination of antioxidant activity of various types of foods using DPPH is comparable to other methods. It is probable each of these methods measure a somewhat different profile of antioxidant compounds.

Antioxidant analysis by other published methods is limited to those compounds soluble in the selected solvent. Antioxidant activity of insoluble compounds was not accounted in a single extraction method. Extraction techniques using different solvents and concentrating the solvent is time consuming. In this method, DPPH is allowed to react with the whole sample. Sufficient time allows DPPH to react slowly with weak antioxidants. The kinetics of flavanones, flavanols and various phenolic compounds scavenging DPPH radicals were studied by electron spin resonance spectrometry and other techniques. Compounds with similar structures follow similar trends using various methods.

Antioxidant activity of grains, dry beans, fresh vegetables and fruits were analyzed using the DPPH method. Some of these results, along with results for some standard antioxidant compounds are presented in Tables 1 and 2. DPPH results for standard phenolic compounds follow similar trends as observed by other methods. Antioxidant content of vegetables and fruits was previously reported using ORAC and other methods (8,9). Similar trends in antioxidant activity were observed for grains, vegetables and fruits comparing those results with those obtained using DPPH. Antioxidant activity of dry beans increases with the red color of the beans, red kidney beans being the highest. Vinson et al. also observed that red kidney beans had higher activity than other beans (4). Similarly, red grapes have higher antioxidant activity than green grapes, and red cabbage is higher than green cabbage. Antioxidant capacity of blueberries and strawberries containing phenolic anthocyanins are high in the range of 3100-5100 TE/100gm. Dried fruits like raisins and dates have high antioxidant activities, 5900-6600 TE/100gm, and these results are consistent with published ORAC data.

Antioxidant activities of various fractions of grain during milling process were studied and the results indicate that bran has the highest antioxidant activity and refined flour had the lowest activity. Antioxidant activities were also studied for ready to eat oat, wheat, corn, and rice cereals. In whole grain cereals, bran and germ are intact, hence antioxidant activity of whole grain cereals is higher than it is in refined grain cereals. Antioxidant activity of whole grain wheat and whole grain oat

cereals is in the range 2200-3600 TE/100gm. Refined grain corn and rice cereals are in the range 1400 - 2000 TE/100gm, as a result of bran removal. Hence, the refined grain cereals have lower antioxidant activity. Similar trends have been observed with whole wheat bread, 2000 TE/100gm versus white bread, 1200 TE/100gm.

CONCLUSION

The antioxidant activity of various foods can be determined accurately, conveniently, and rapidly using DPPH. The trend in antioxidant activity obtained by using the DPPH method is comparable to trends found using other methods reported in the literature. This method can be used successfully for solid samples without prior extraction and concentration, which saves time. The reaction time of four hours and a temperature of 35°C facilitates the extraction and reaction of antioxidant compounds with DPPH. Antioxidant activity measured using DPPH accounts partially for the bound and insoluble antioxidants. Relative antioxidant content provides an indication of importance of each of the foods. Antioxidant activity and nutritional labeling data including vitamins, fibers, minerals will aid in the interpretation of clinical results obtained as various food products are tested in biological models for chronic disease. It is reasonable to expect that high antioxidant foods have greater potential to reduce free radicals in the body than do low antioxidant foods. Thus it is important to know the antioxidant content of foods, in addition to knowing the basic nutritional information such as the protein, fiber, mineral and vitamin contents.

A recent study conducted by scientists at the University of Texas Medical Branch at Galveston (UTMB) showed that the curcumin pigment in the curry spice prevents colorectal cancer. Numerous previous studies have already acknowledged the beneficial properties of the natural pigment against a wide range of cancers, including colorectal one. But the current study is based on curcumin's potency to block the neurotensin gastrointestinal hormone, which is closely connected to the production within our bodies of an inflammatory protein which plays a key-role in the growth and proliferation of cancer cells. The study carried out by UTMB researchers is entitled 'Curcumin inhibits neurotensin-mediated interleukin-8 production and migration of HCT116 human colon cancer cells'. The team stated they hope that further studies on the idea of the current

research will develop new strategies of fighting against colorectal cancer, by using curcumin to inhibit the production of the neurotensin hormone. Mark Evers, senior author of the study and director of UTMB's Sealy Center for Cancer Cell Biology pointed out: “Our findings suggest that curcumin may be useful for colon cancer treatment, as well as potential colon cancer suppression, in cells that respond to this gastrointestinal hormone, neurotensin. About a third of all colorectal cancer cells have the receptor for neurotensin.” He also explained: “We found that in colon cancer cells, neurotensin increases not just the rate of growth but also other critical things, including cell migration and metastasis. The fact that all that can be turned off by this natural product, curcumin, was really remarkable.” Curcumin is the yellow pigment found in the curry spice turmeric. Turmeric is commonly known as one of the cheapest spices, that has been used since antiquity as a condiment and also as dye. Originating in South East Asia, the turmeric contains curcuminoids that are the most beneficial ingredients of this plant. Previous researches found that curcuminoids have antioxidant, antibacterial and anti-inflammatory effects upon human body. Also, the turmeric intake helps supporting the health of the liver, prevents high doses of cholesterol in our organism and is being studied by scientists for its tumor-blocking ability. As an anti-cancer agent, turmeric has been found to block the uncontrollable growth of melanoma tumor cells, cells that lead to breast And colon cancer, leukemia etc.

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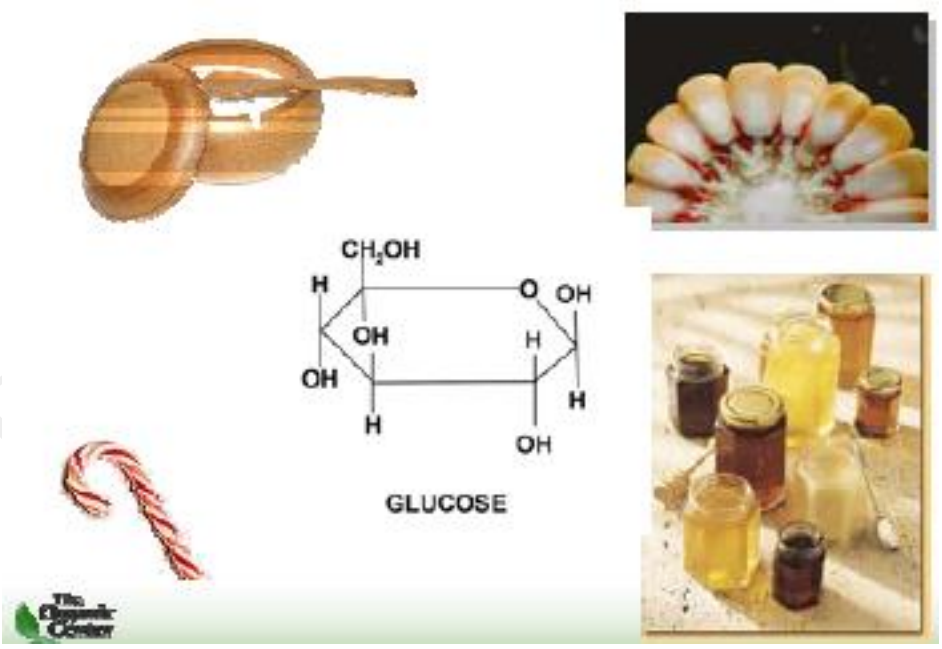
IV. ANTIOXIDANTS: THERAPEUTIC BASE FOR TREATMENT OF POLYGENIC DISORDERS

IV. Antioxidants: Therapeutic Base for Treatment of Polygenic Disorders

Hyperphysiological burden of free radicals causes imbalance in homeostatic phenomena between oxidants and antioxidants in the body. This imbalance leads to oxidative stress that is being suggested as the root cause of aging and various human diseases like atherosclerosis, stroke, diabetes, cancer and neurodegenerative diseases such as Alzheimer's disease and Parkinsonism. Therefore, in modern Western medicine, the balance between antioxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system. Researches in the recent past have accumulated enormous evidence advocating enrichment of body systems with antioxidants to correct vitiated homeostasis and prevent the onset as well as treat the disease caused /fostered due to free radicals and related oxidative stress.

This article presents current understanding of the role of free radicals and oxidative stress in pathogenesis of various diseases and advancements made in developing antioxidant based therapeutics and also discuss the opportunities to develop therapeutics from traditional medicinal practice.

ANTOINE Lavoisier, a pioneer oxygen chemist, had pointed out about 150 years ago that animals that respire are true combustible



bodies that burn and consume themselves1. The biological combustion produces harmful intermediates called free radicals. A free-radical is simply defined as any species capable of independent existence that contains one or more unpaired

electrons, an unpaired electrons being one that is alone in an orbital. It may be superoxide (O2 ·–, an oxygen centred radical), thiyl (RS·, a sulphur-centred radical), trichloromethyl (CCl_3 · , a carbon centred radical) or nitric oxide (NO·) in which the unpaired electron is delocalized between both atoms. The O_2 ·–, hydroxyl radicals (·OH) and other reactive oxygen species (ROS) such as H_2O_2 are continuously produced *in vivo*. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and our metabolism. They are continuously produced by the body’s normal use of oxygen such as respiration and some cell-mediated immune functions. These free radicals are also generated through environmental pollutants, cigarette smoke, automobile exhaust fumes, radiation, air pollutants, pesticides, etc.2. These exogenous pollutants generating free radicals, have become part and parcel of our daily inhaling/ingesting life and infact there appears no escape from them. Continuous interaction of the animal physiological systems with these free radicals generated either indigenously or inhaled/ingested from exogenous sources therefore, lead to excess load of free radicals and cause cumulative damage of protein, lipid, DNA, carbohydrates and membrane, resulting in so-called oxidative stress. Therefore, living creatures have evolved a highly complicated defence system with antioxidants composed of enzymes and vitamins against oxidative stress in the course of their evolution. These defence systems are mainly classified3 as (i) suppression of generation of ROS, (ii) scavenging of ROS, (iii) clearance, repairing and reconstitution of damage and (iv) induction of antioxidant proteins and enzymes.

However, amounts of these protective devices present under normal physiological conditions are sufficient only to cope with the normal threshold of physiological rate of free-radical generation. Therefore, any additional burden of free radicals, either from an indigenous or exogenous source on the animal (human) physiological system can tip free radical (prooxidant) and anti-free radical (antioxidant) balance leading to oxidative stress2. The oxidative stress, defined as the imbalance between oxidants and antioxidants in favour of the former potentially leading to damage has been suggested to be the cause of aging and various human diseases4. Therefore, in modern Western medicine, the balance between antioxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system5,6. Any vitiation therefore, is understood to give rise to disorderliness in the physiological system leading to a variety of diseases depending upon the sensitivity and susceptibility of the organ. Thus, the status of protective mechanism against oxidants, the antioxidants in humans reflect the dynamic balance between antioxidant defence and prooxidant conditions and have been suggested as a useful tool in estimating the risk of oxidative damage2,7–9.

Research in the recent past has accumulated enormous evidences revealing that enrichment of body systems with natural antioxidants may correct the vitiated homeostasis10– 13 and can prevent the onset as well as treat diseases caused and/or

fostered due to free-radical mediated oxi dative stress. These developments accelerated the search for antioxidant principles that lead to the identification of natural resources, isolation of active principles and further modification and refinement of active antioxidant molecules. However, animal experimentations and clinical evaluations with these active ingredients were not only punctuated by some success, but also by some spectacular failures2. Nevertheless, success and failures with these efforts and concurrent advancement in understanding of free-radical biology and related pathogenesis provided important insights in further improving the development of newer molecules/molecular compositions that may provide better preventive and therapeutic potentials. The perpetual tendency of learning towards refinement of understanding and the knowledge, has now led to the discovery and development of several novel antioxidant based molecules/drugs/natural medicinal

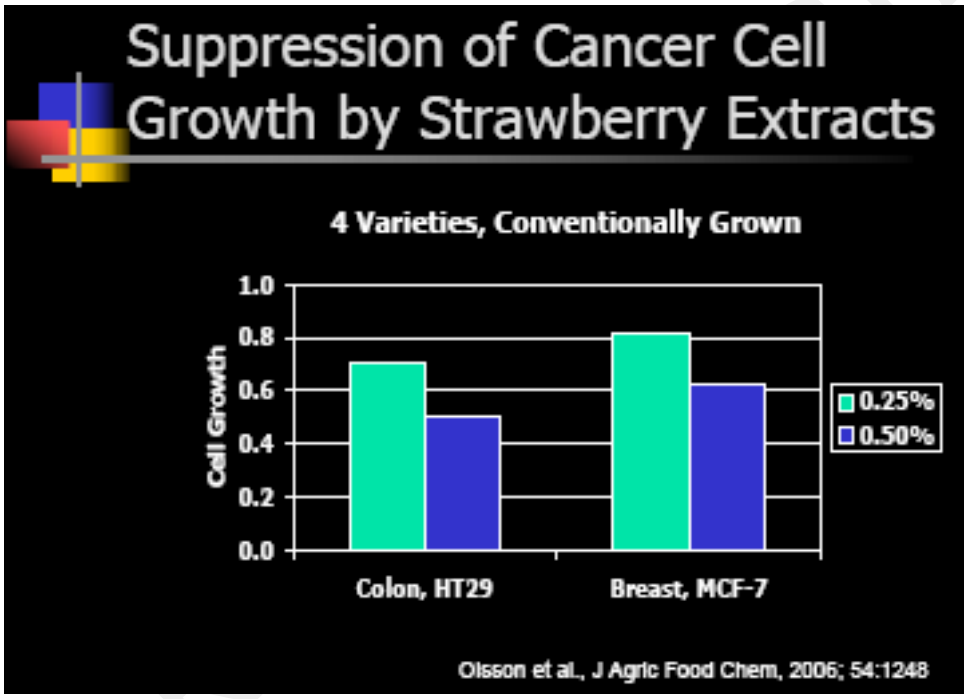


Fig.1. Suppression of Cancer Cells.

compositions that have satisfied majority of the aspects of complex pathogenic steps in diseases for which only disease/risk factor-modifying therapies are available. This article presents a general account on understanding the role of oxidative stress and efforts made in developing novel antioxidant-based drugs/formulations for prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer’s disease, Parkinson’s disease, cancer, etc. and discusses the scope for further strategic development of newer therapies.

Antioxidants

In foods, antioxidants have been defined as a substance that in small quantities is able to prevent or greatly retard the oxidation of easily oxidizable materials such as fats14. However, in biological systems the definition for antioxidants has been extended to any substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate like lipids, proteins, DNA, and carbohydrates15. Currently however, biological antioxidants have further assumed a broad definition to include repair systems such as iron transport proteins (e.g. transferrin, albumin, ferritin and caeruloplasmin), antioxidant enzymes, and factors affecting vascular homeostasis, signal transduction and gene expression16.

Antioxidants may exert their effects by different mechanisms, such as suppressing the formation of active species by reducing hydroperoxides (ROO·) and H2O2 and also by sequestering metal ions, scavenging active free radicals, repairing and/or clearing damage. Similarly, some antioxidants also induce the biosynthesis of other antioxidants or defence enzymes. The bioactivity of an antioxidant is dependent on several factors like their structural criteria, physico-chemical characteristics and *in vivo* radical generating conditions (see ref. 2 and references cited therein). An antioxidant works by retarding the oxidation. In biology, oxidation is often started by free radicals. The role of an antioxidant is to intercept a free radical before it can react with the substrate. For example, phenol (AOH),
the reaction of interest with ROO· is:

$$AOH + ROO\cdot \longrightarrow AO\cdot + ROOH.$$

This H-atom transfer reaction effectively stops chain reaction. Therefore, antioxidants of biological/therapeutic importance should have the property that they will react/ trap the free-radical before it reacts with the susceptible substrate and initiate chain reaction. Based on several theoretical models and complex calculations, Wright17 concluded that bond dissociation enthalpy (BDE) gives excellent correlation for this requirement with many known families of antioxidants that have been extensively studied in biological systems, like vitamins E and C, resveratrol, gallocatechins, ubiquinol, etc. He suggested that lower the BDE, the more reactive the antioxidant. However, it should not be too low to reduce the molecular oxygen, forming HO2 · the process of autoxidation17.

This imbalance can be managed by exogenous supply of antioxidant rich nutrition, natural and/or synthetic antioxidant principles- based therapeutic preparations.

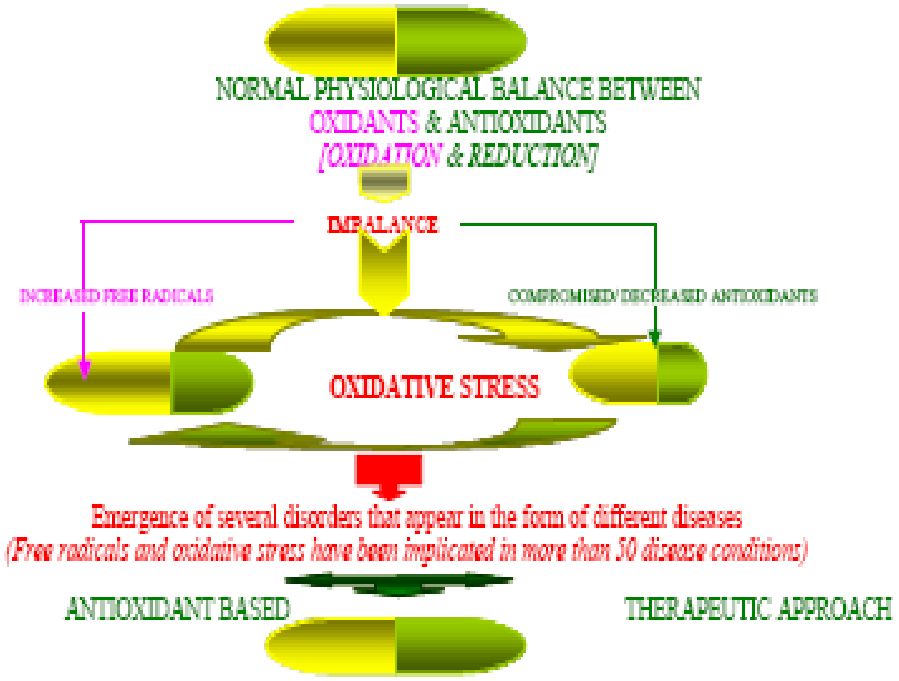


Figure 2. Oxidative stress occurs due either to the increased generation of free radicals or compromised and/or decreased antioxidant defence.

Major understanding of beneficial therapeutic activities of antioxidants has arisen with studies on vitamins E and C and ubiquinol Q10 that serve as excellent reference material. Mechanisms of radical scavenging activity of antioxidants and their pros and cons are well available2,10. The most active component of vitamin E, *a*-tocopherol, is a good lipid-soluble, chain-breaking antioxidant that breaks the cycle of lipid peroxidation by reacting with ROO· before it can attack lipid molecules. Another excellent antioxidant is water-soluble vitamin C. Vitamin C is used to repair vitamin E radical10. Based on BDE calculations of these important antioxidants, Wright17 proposed a design window for an antioxidant to be ideal. The useful design window for an ideal antioxidant based on BDE calculations may be in the range of 68–76 kcal mol–1, i.e. higher than vitamin C and lower than *a*-tocopherol. Based on various drug-discovery groups and programmes the world these considerations, it has been made possible to design several antioxidant molecules that have displayed excellent biological activities. Simultaneously, it was also observed that majority of antioxidants originating from natural products fall under this criterion17.

This understanding may provide future directions for design, synthesis and also search, identify and prioritize novel antioxidants from natural resources. Progress in sciences is providing crucial insights in the understanding mechanisms of disease pathogenesis and is opening up rich field of potential targets for pharmaceutical

intervention. The broader and clearer understanding of the molecular basis of disease processes therefore is paving a way to develop more effective and targeted treatment. Over the past three decades, free-radical theory has greatly stimulated interest in the role of dietary antioxidants in preventing many human diseases, including cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration and diabetes (ref. 18 and references cited therein).

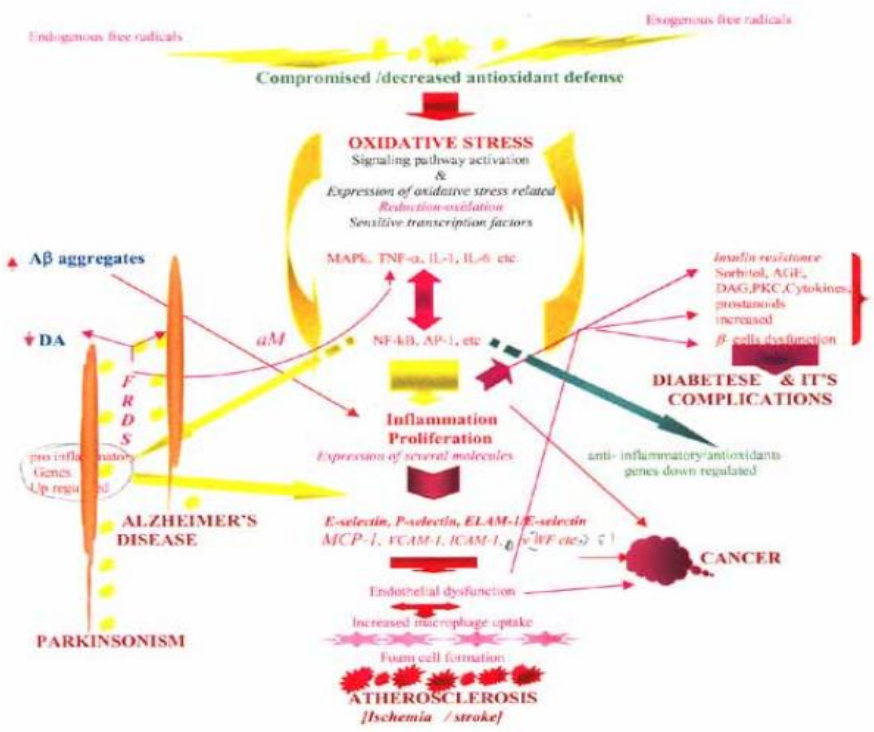


Figure 3. Involvement of free radicals and oxidative stress-induced expression of *red-ox* sensitive factors, cytokines and adhesion molecules leading to the development of various diseases.

These wide varieties of chronic inflammatory diseases form the basis for development of antioxidant based therapeutics. Regardless of their initiating pathological events, these diseases share a series of steps that lead to a common mechanistic pathway of oxidative stress through regulatory oxidative signals19 (Figure 3).

Free radicals act as signalling intermediate and initiate receptor-mediated activation of intracellular signalling pathways that activate the production of inflammatory chemokines and cytokines. MAPk cascades are activated by various free radicals, cellular stressors, and growth factors and are involved in several biological responses like cytokines production, differentiation, proliferation and cell death. TNF exerts a

variety of biological effects like production of inflammatory cytokines, up-regulation of adhesion molecules, proliferation, differentiation and cell death. It induces free radicals accumulation and also acts as a strong activator of NF-kB. NF-kB is a transcriptional factor that regulates expression of various inflammatory cytokines, chemokines and adhesion molecules, and plays an important role in vascular cell functions. A*b*, Amyloid beta protein; AGE, Advanced glycation end-products; aM, Activated microglia; AP, Activator protein; DA, Dopamine; DAG, Diacylglycerol; ELAM, Endothelial leukocyte adhesion molecule; FRDS, Free radicals; ICAM, Intracellular-adhesion molecule; IL, Interleukin; MAPk, Mitogenactivated protein kinase; MCP-1, Monocyte chemoattractant protein; NF-kB, Nuclear factor-kappa B; PKC, Protein kinase C; TNF, Tumour necrosis factor; VCAM, Vascular cell-adhesion molecule; vWF, von Willebrand factor. Details in the text and references cited appropriately at respective places.

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over have excelled in designing and developing novel antioxidant- based drug molecules that have proven their therapeutic efficacy and have gathered information for further advancements in designing and developing drug molecules for treatment of diseases where no satisfactory therapy is available.

Atherosclerosis

Oxidative stress, especially oxidation of low-density lipoproteins (LDL), has long been suspected to play a critical role in atherogenesis, in consequence of which antioxidants were expected to have antiatherogenic potential. Such agents were thought to be able to inhibit oxidative modification of LDL that leads to the accumulation of cholesterol in the atherosclerotic lesion20–22. Furthermore, insights gained from genomic analysis of both the oxidized lipids and antioxidants have further changed the perspective of the etiology of this complex disease. The findings by genomic analysis provide key elements for molecular mechanisms that contribute to the antiatherosclerotic properties of phenolic antioxidants.

Differences in gene expression in various animal models and in different regions of the aorta in response to these antioxidants may be one reason that antiatherogenic effects vary greatly depending upon the animal model and site of disease progression23. It was observed that high fat diet increased 32 genes; however, only three genes were observed to be related to atherogenesis. On the other hand, some of the genes showing decreased expression were those of free-radical scavenging enzymes, resulting in increase of oxidative stress, which was attenuated by antioxidant supplementation24. These observations support the view that hyperlipidemia is merely a risk factor and oxidative stress is the *root cause* of atherogenesis. Emerging evidence therefore, for diverse functions of both the phenolic antioxidants and oxidized lipids at molecular levels, clearly guides for development of global systems approach. However, for those actively engaged in developing potential therapeutics, clinical trials of antioxidants have given rise to

some controversies regarding their real clinical benefits25–27. Though several compounds classified as antioxidants have been shown to have antioxidant properties, probucol23 and vitamin E as *a*-tocopherol28 happened to be the pioneer antioxidant candidates that have made basis for the understanding of their mode of action and provided substantial insights in further designing and developing novel antioxidant-based molecules, encompassing important structural/functional features to impart better therapeutic potential and also overcome the unwanted effects.

Many therapeutic agents have been developed to counteract major risk factors for cardiovascular disease like hyperlipidemia and hypertension. However, no therapy is available to address the root cause of atherosclerosis. Recently, two groups have emerged with success in designing, developing and taking antioxidant-based antiatherosclerotic candidates to the level of clinical-trials28,29. The ultimate clinical success of these candidates (Figure 3) will no doubt revolutionize the therapy of atherosclerotic diseases. Similarly, several therapeutic preparations from traditional medicines like *Terminalia arjuna*30,31, Abana32,33, Campo medicine34, and MAK35 may have significant therapeutic potential to address antiatherosclerotic properties in clinical settings.

Stroke

As recently as five years ago, most physicians would have confidently described atherosclerosis as a straight plumbing problem. In atherogenesis, fat-laden gunk gradually builds up on the surface of passive artery walls and when the deposit (plaque) grows large enough, it eventually closes off an affected 'pipe', preventing blood from reaching its intended tissue; after a while, the blood starved tissue dies. As a result, when a part of the cardiac muscle succumbs, it leads to heart attack and when it affects the brain it is called a stroke. Many strokes stem instead from less obstructive plaques that rupture suddenly, triggering the emergence of a blood clot, or thrombus, that blocks blood flow36.

Stroke is the third leading cause of death and the major cause of disability in USA. In the general population, incidence of stroke is 1/1000 individuals, however, incidence doubles in individuals who are 80 years of age37. Stroke is defined as an abrupt impairment of brain function resulting from occlusion or rupture of intra or extra cranial blood vessels. There are several types of stroke:

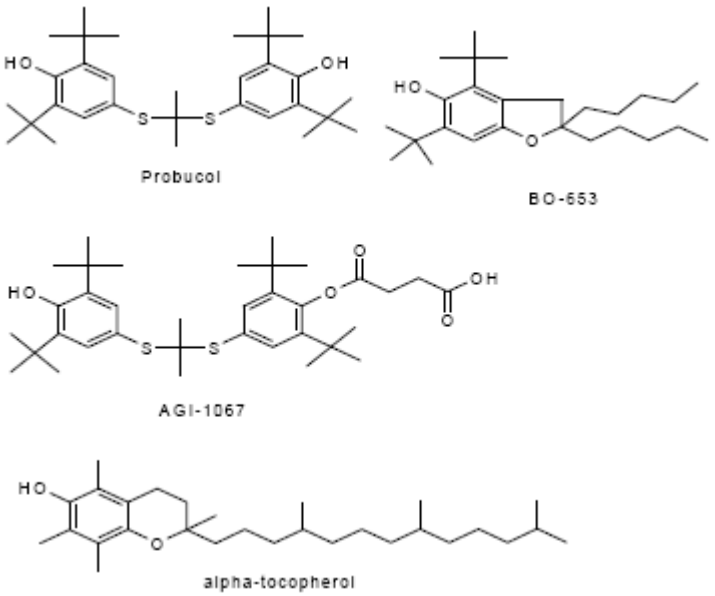

Figure 4. Structure of important compounds discussed in the literature.

BO-653 and AGI-1067 are the recent developments up to clinical trial as antiatherosclerotic antioxidants. Probucol and alpha tocopherol serve as the basis for the design and development of these compounds. cerebral thrombosis and cerebral embolism, also classified as ischaemic stroke and subarachnoid haemorrhage, and intracerebral haemorrhage also classified as haemorrhagic stroke.

Cerebral thrombosis is the most common type of stroke and occurs when a thrombus develops on the wall of a cerebral artery, usually damaged by atherosclerosis37. Therefore, therapeutics developed for atherosclerosis, based on imbalance between oxidant and antioxidant homeostasis appears to be important in treating stroke provided it reaches the brain-tissue sites. Extensive research generated in the recent past has disclosed that free radicals play a major role in the damage caused by hypoxia and reperfusion during cerebral ischaemia, affecting a late stage of the ischaemic process. These developments also support views that agents that scavenge free radicals or prevent their production may be able to prolong the therapeutic time window37. Several antioxidants and freeradical scavenging-based therapeutics have been recently launched and are under development for treatment of stroke37.

Diabetes and diabetic complications

There is considerable evidence that hyperglycemia results in the generation of ROS, ultimately leading to increased oxidative stress in a variety of tissues. In the absence of an appropriate compensatory response from indigenous antioxidant network, the system becomes overwhelmed (redox imbalance), leading to the activation of stress sensitive intracellular signalling pathways. One major consequence of this is the

expression of gene products that cause cellular damage and are ultimately responsible for late diabetic complications (Figure 2). Apart from playing a key role in late diabetic complications, activation of it or similar signalling pathways also appears to play a role in mediating insulin resistance and impaired insulin secretion. The ability of antioxidant/free-radical scavengers to protect against the effects of hyperglycemia and free fatty acids along with clinical benefits following antioxidant therapy, supports the causative role of oxidative stress in mediating and/or worsening these abnormallities38.

A number of reviews have appeared recently, stressing the role of oxidative stress in pathogenesis of cellular dysfunction leading to cardiovascular, hepatic and other complications of diabetes38–40. Similarly, supplementation with antioxidants has also been shown to decrease oxidative stress and complications in animal models of diabetes41,42 and diabetic patients43. Diabetes-induced defects in the homeostasis and the transport of intracellular calcium have been shown to decrease or recover by treatment of diabetic animals with some antioxidants44. Several studies have demonstrated that antioxidants supplementation prevents lipid peroxidation, haemoglobin glycation and inhibition of Na+, K+- ATPase and/or Ca++-ATPase activity caused by hyperglycemia in various cells45,46.

Stobadine is a synthetic drug and scavenges a variety of free radicals (ref. 44 and references therein). Blood glucose-lowering effect of stobadine treatment in streptozotocine (STZ)-induced diabetes in animals starts two days after the STZ injection and its effect has been directly correlated to its free-radical scavenging properties, which may protect pancreatic *b*-cells against STZ toxicity47.

Stobadine in low dose is able to lower blood glucose and tissue calcium accumulation in STZ-diabetic rat. It has also been observed that together with vitamin E, it can provide better control on hyperglycemia-induced oxidative stress44. Multiple activities of phytochemicals present in traditional medicines and their preparations have been reviewed recently48. There are several medicinal plants the world over used in traditional medicine, which possess rich antioxidant principles and strong antioxidant activities. It has been argued that major antidiabetic activities from these plants might originate from their antioxidant principles49,50. Taking the advantage of modern drugs like stobadine and its detailed mechanism of action, natural medicines may also be developed explaining their therapeutic properties and mechanism of action. These efforts may provide novel mechanism-based application of traditional medicines used in this disorder.

Neurodegenerative diseases

Aging is the major risk factor for neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD). Oxidative stress may induce neuronal damage, ultimately leading to neuronal death by apoptosis or necrosis. A large body

of evidence indicates that oxidative stress is involved in the pathogenesis of AD and PD. Simultaneously, increasing number of studies show that nutritional antioxidants can block neuronal death and may have therapeutic properties in animal models of these neurodegenerative diseases51,52.

Alzheimer's disease

The risk of acquiring AD is considerable in countries with long life expectancies. Projected national burdens related to AD are daunting as unprecedented numbers of people are expected to survive their eighth and ninth decade of life. In USA alone, the current estimate of 3.6 lakh new cases of AD each year is expected to triple in the next 40 years53. AD is the commonest form of dementia with a prevalence of 0.4% in women and 0.3% in men aged 60–69 years54. Estimated prevalence of senile dementia in Europe increases with age from 1% in man and women of age 60 years to 44.7% in a population of 90–95 years age55.

Selective sensitivity and vulnerability of neurons are the most important characteristics of this disorder. Freeradical theory of aging suggests that oxidative damage is a major player in degeneration of cells56. The role of oxidative stress in the etiology of AD has long been hypothesized, described, and supported by a variety of experimental and clinical studies. This research has also promoted interest in assessing antioxidants for their possible benefits in modifying the course, reducing the risk, or delaying the onset of AD. Recent research reveals that dietary antioxidants may have promising therapeutic potential in delaying the onset as well as preventing the aging population with AD and its related complications57. Characteristic histopathological alterations in AD are neutristic plaques composed largely of amyloid *b*-peptides (A*b*) and neuronal aggregates of abnormally phosphorylated cyctoskeletal proteins52. Several lines of evidence (ref. 51 and references cited therein) suggest that over production of ROS is implicated in A*b* neurotoxicity: (i) exposure of cultured neurons or neuronal cell lines to A*b* increases intracellular levels of ROS leading to the activation of NF-kB; (ii) markers of oxidative stress are found increased in transgenic mouse models of AD; (iii) neurotoxicity of A*b* is attenuated by antioxidants such as vitamin E, PBN (*a*-phenyl-tert-butylnitrone), lazaroids and free-radical scavengers.

A controlled clinical trail with DL-*a*-tocopherol and selegiline in patients with moderately severe impairments of AD has shown some beneficial effects with respect to the rate of deterioration of cognitive function58. Vitamin E has been proposed to impart beneficial effect in this connection by quenching the ROS formed, and selegiline protects neurons by preventing the formation of ROS and by inhibiting oxidative metabolism of catecholamines. These advances provide a sound basis for search, design and development of targetted antioxidants for prevention and treatment of AD.

Parkinson's disease

PD is a neurological syndrome manifested by any combination of tremor at rest, rigidity, bradykinesia and loss of postural reflexes. Neuropathalogical hallmark of PD is selective degeneration of dopaminergic neurons in the nigrostriatal system59. These neurons synthesize and release dopamine (DA), and loss of dopaminergic influence on other structures in the basal ganglia leads to classical Parkinsionian symptoms51.

Epidemiological studies indicate that a number of factors like exposure to herbicides, industrial chemicals, trace metals, cyanide, organic solvents, carbon monoxide and carbon disulphide may increase the risk of developing PD60. Majority of them are known to increase ROS and oxidative stress. Oxidative stress may arise from the metabolism of DA with the production of potentially harmful free radicals61. Alterations in pro- and antioxidant molecules have also been observed in post-mortem tissues from individuals with PD51. Activated microglia (aM) are thought to contribute to neuronal damage via the release of proinflammatory and neurotoxic factors like TNF*a*, IL-1, RNS, and ROS, etc. The reactive free radicals and their downstream products have been shown to contribute substantially to the oxidative damage in PD. Markers of elevated accumulation of NO, ROS, TNF*a*, IL-1*b*, INF-*g* in substantia nigra of PD patients have been demonstrated62.

Two neuroprotective clinical trials are available with antioxidants: (i) Deprenyl and tocopherol antioxidant therapy of Parkinson's study observed that deprenyl63, an antioxidant molecule and also MAO-B inhibitor slowed early progression of symptoms and delayed the emergence of disability by an average of nine months. However, vitamin E at a given dose could not display significant effects.

(ii) But in another open trial, combination of high dosage of *a*-tocopherol and ascorbate delayed the emergence of disability by 2.5 years, the time necessary to begin therapy with L-DOPA64. Esposito *et al*.51 suggest that there are many alternative antioxidative approaches that may be considered in future clinical trials, including free radical scavengers, indigenous antioxidant enzyme boosters, iron chelators and drugs that interfere with oxidative metabolism of DA in Parkinsonism.

CANCER

The recent world cancer report released by WHO observes that world cancer rates are set to double by 202065. Cancer is emerging as a major problem globally; both in more developed and in less developed countries. Furthermore, cancer mortality in the world as a whole is more than twice that in developing countries, a factor the report attributes to the earlier onset of the tobacco epidemic, earlier exposure to occupational carcinogens and the western diet and life style. Carcinogenesis is a multistage disease process that has been classified into initiation, promotion and progression stages; and each stage probably involves both genetic and epigenetic

changes66. These observations have been substantiated experimentally by external administration of carcinogens67. Metabolic activation of carcinogen is a free-radical-dependent reaction. DNA damage mediated by free radicals plays a critical role in carcinogenesis68,69. In biological systems, damaged DNA is repaired enzymatically and cells regain their normal functions. However, misrepair of DNA damage may result in mutations such as base substitution and deletion, leading to carcinogenesis70. Sequence specificity of DNA damage plays a key role in the mutagenic process. Endogenous DNA damage arises from a variety of intermediates of oxygen reduction and several free radicals have been reviewed to take part in this process by various mechanisms71. These reactive species have different redox potentials and redox potentials of these free-radical species may play an important role in sequence- specific DNA damage17. Apart from redox-potential of free-radical species, oxidation potential of DNA bases also contributes to the determination of sequence specificity of DNA damage.

Guanine is most easily oxidized among the four DNA bases, as its oxidation potential is lowest (1.29 V vs normal hydrogen electrode) among others (adenine 1.42 V, cytosine 1.6 V and thiamin 1.7 V)72,73. Though the most common hydroxyl radical causes DNA damage with no marked site specificity, Kawanishi *et al*.71 have delineated the mechanism of guanine-specific DNA damage by different free-radical entities and their role in carcinogenesis.

Apart from a variety of free radicals, non-radical oxidant like H_2O_2 also play an important role in DNA damage. In biological systems, H_2O_2 is generated through spontaneous and/or superoxide dismutase (SOD) catalysed dismutation of $O_2 \cdot$. The $O_2 \cdot$ is produced by one electron reduction of molecular oxygen through reaction with free radicals and enzymatic reaction catalysed by xanthine oxidase. H_2O_2 has emerged as a pivotal molecule not only for cancer cell proliferation, but also in determining the fate of cancer cells exposed to phenolic phytochemicals. Higher amounts of ROS and H_2O_2 are produced in some cancer cells. The cumulative production of free radicals and H_2O_2 in human melanoma, neuroblastoma, colon carcinoma and ovarian carcinoma cell lines are comparable to that in phorbol ester-stimulated human blood neutrophils74. Since cancer cells constitutively produce high amounts of H_2O_2, the concept of persistent oxidative stress in cancer originated75, which provides plausible explanation for some of the abnormal characteristics of cancer cells76.

Recently, Loo76 has reviewed redox-sensitive mechanisms of phytochemicals (particularly antioxidant polyphenols) mediated inhibition of cancer cell proliferation. Cancer cells, particularly those that are highly invasive or metastatic, require a certain level of oxidative stress to maintain a balance between undergoing either proliferation or apoptosis. They constitutively generate large but tolerable amounts of H_2O_2 that apparently function as signalling molecules in mitogen-activated protein kinase (MAPK) pathway to constantly activate redox-sensitive

transcription factors and responsive genes that are involved in survival of cancer cells as well as their proliferation. With such a reliance of cancer cells on H_2O_2, it follows that if the excess H_2O_2 can be scavenged by phenolic phytochemicals having antioxidant activity, the oxidative stress-responsive genes can be suppressed and consequently proliferation of cancer cells can be inhibited. On the other hand, phenolic and other phytochemicals known as isothiocyanates, can induce the formation of H_2O_2 to achieve an intolerable level of high oxidative stress in cancer cells. As an early response, stress genes are activated; however, when critical threshold for cancer cells to cope with the induced oxidative stress reaches beyond tolerable limits, the key cellular components such as DNA suffer irreparable damage. In conjunction, genes involved in initiating cell-cycle arrest and/or apoptosis get activated. Therefore, the antioxidant phytochemicals can either scavenge constitutive H_2O_2 or paradoxically generate additional amounts of H_2O_2 to inhibit proliferation of cancer cells76 and act as anticancer agents. Apart from these actions, antioxidants have also been advocated to impart anticancer activities by several other mechanisms77: (i) Trapping the ultimate carcinogen, (ii) blocking the metabolic activation of carcinogens, (iii) modulating xenobiotic metabolizing enzymes, (iv) scavenging free radicals, (v) inhibiting generation of free radicals, (vi) inhibiting promotion stage of carcinogenesis by inhibiting cell proliferation through blocking lipoxygenase/ cycloxygenase pathway or by lowering ornithine decarboxylase activity, and (vii) by decreasing the bioavailability of ultimate carcinogen, etc.

Insights towards developing novel antioxidants

The above discussions throw light on the involvement of some common pathways in pathogenesis of different diseases mediated through oxidative stress and free radicals (Figure 3). It has been observed there is some commonality in antioxidant molecules, i.e. presence of phenolic pharmacophore3,23 (Figure 4).

Indeed, various kind of radicals like hydroxyl, alkoxyl, peroxyl and carbon centred, have been observed to be involved in oxidative stress *in vivo*, Niki *et al.*78 suggest that considering the activities of radicals and concentration of substrates, the peroxyl radicals should be the major target radical for radical scavenging antioxidants *in vivo*. Activity of phenolic antioxidants towards peroxyl radicals is determined primarily by BDE of phenolic O–H bond, its redox potential and steric hindrance to abstraction of phenolic hydrogen by peroxyl radicals79,80. Peroxyl radicals are electrophilic in nature. Therefore, electrondonating substituents on the aromatic ring of the phenolic pharmacophore increases the reactivity towards peroxyl radical, whereas electron-withdrawing groups decreases it. In addition to such polar effects, the electron-donating substituents also weaken phenolic O–H bond, while electron-withdrawing substituents increases O–H bond dissociation energies. Therefore, it is important that an antioxidant should have electron-donating substituents77. Furthermore, substituents on the ortho-position are important in determining the stability of phenoxyl radical, hindrance for approach of peroxyl radicals to the

phenolic hydrogen, and the reactivity of phenoxyl radical towards the substrate81. Flavanols, present in abundance in dietary constituents, contain a strong nucleophilic centre that reacts with electrophilic species and thereby decreases the bioavailability of the ultimate carcinogens82. Therefore, presence of nucleophilic electron-donating properties of polyphenols present in tea may be one of the important mechanisms of action involved in inhibition of carcinogenesis, where electrophilic carcinogenic species may be trapped by nucleophilic polyphenols77.

Considering these criteria and antioxidant property as the base in view, several novel molecules have emerged for development as therapeutics in various multifactorial diseases like atherosclerosis23,28,29,83,84, stroke37, cancer17,76, and simultaneously, several insights have been provided for therapeutic advancement for AD and PD51,52. Diphenyl picrylhydrazyl (DPPH) is a nitrogen-centred free radical. It reacts similar to the peroxyl radical. Its reaction rates correlate directly with antioxidant activity. Higher the rate, more effective the antioxidant17. Two mechanisms for antioxidants to scavenge DPPH radical have been proposed85: the first is a direct H-atom abstraction process (eq. (1)) and the second is a proton concerted electron-transfer process (eq. (2))

$$DPPH\cdot + RXH \longrightarrow DPPHH + RX\cdot, \quad (1)$$

$$DPPH\cdot + RXH \longrightarrow DPPH^{-} + RXH^{\cdot +}$$
$$\longrightarrow DPPHH + RX\cdot. \quad (2)$$

In which, X represents either O, N, S or C. First pathway is governed to a large extent by X–H BDEs, of RXH and DPPHH. Only if the BDE of former is lower than that of the latter, the reaction is permitted. The BDE for DPPHH is calculated to be 172.22 kcal/mol. While, the second pathway is determined by ionization potentials (IP) of RXH and DPPH–. The prerequisite for this reaction to proceed is that IP of the RXH should be lower than that of DPPH–. The IP for DPPH-is observed to be 59.60 kcal/ mol. Phenol, amino, or thiophenol groups are commonly known to be the active groups for scavenging DPPH. These understandings may provide better insights in designing the active centers for antioxidants as radical scavenger and by protecting these centers, further modify structures to improve the absorption and metabolic properties in the molecule.

Edaravone is a novel neuroprotective agent approved for acute therapy of embolic stroke. The pharmacological effect of it arises from its radical scavenging activity. However, it does not possess phenolic pharmacophore. It has been observed recently that edaravone scavenges DPPH through donating H-atom85, as the C–H BDEs of edaravone (77.26–86.36 kcal/mol) are much lower than DPPHH. However, it cannot scavenge DPPH by second method as its IP (164.72 kcal/mol) is much higher than DPPH–.Antioxidants tested on DPPH were also found extremely effective in cell systems of oxidative stress used to test anticancer agents17. This simple test further provides information on the ability of a compound to donate electrons, the number of

electrons a given molecule can donate and on the mechanism of antioxidant action. Furthermore, in cases where the structure of the electron-donor is not known (e.g. a plant extract), this method can afford data on the reduction potential of the sample, and hence can be helpful in comparing the reduction potential of unknown materials. Vaya *et al*.86 observed that compounds which were able to donate electrons to the DPPH molecule were the same as those that showed high activity in inhibiting LDL oxidation induced under different conditions. Similarly, among several mechanisms for the development of diabetic complications, increased level of advanced glycation end-products (AGEs) are well known to be a cause of aging and diabetic complications. Matsuda *et al*.87 and Lou *et al*.88 have observed that the AGEs formation inhibitory activities of several flavonoids were in accordance with their DPPH radical scavenging activities.

Therefore, this simple test model may be helpful in identifying antioxidant resources as well as molecules useful for development of anticancer, antiatherosclerotic, antidiabetic therapeutics and neuroprotective agents. Applying this model as prerequisite, it has been possible for us to identify several traditional medicinal preparations and medicinal plants bearing rich content of antioxidants89, isolate a number of molecules90–93. Furthermore, we also improved and incorporated several biological activities in that molecules92. These medicinal plants have been used for a variety of disease conditions now being explained by oxidative stress theory. These resources/ medicinal plants therefore, may provide an important base and molecules as building blocks for development of indigenous therapies for disease of polygenic origin.

Future scenario

Technology-based economic growth has been one of the prime factors in creating the wealth of a nation. The most developed countries are characterized by their wealth creation based on pursuing high quality research and development investments and translating their innovations into commercial products94. These criteria appear tough for the developing nations, as they are poorly prepared to invest large sums of money for advanced research and development. Therefore, developing countries like India may find a solution by looking back into their glorious past of traditional medicinal practice like Ayurveda, Unani and Siddha for alternative therapeutic options. Ayurveda and Siddha, discovered, nurtured and perfected in India as science of longevity, are not just a collection of therapeutic recipes, but also frameworks that define the condition of sickness and connect them with healing practices. In olden days these scientific disciplines not only thrived in India but also influenced healing practices in many other countries. That period of intense creativity was a glorious one and every Indian has the reason to remember it with pride95. However, after January 2005, when new intellectual property regulations come into force, Indian companies may no longer copy drugs, but will have to develop them on their own. As an alternative therefore, they may rely on the traditional medical knowledge and

biodiversity as springboards. By fusing ancient wisdom and modern science, India can create world-class products96. Therefore, it has embarked on a fast track programme to discover new drugs by building on traditional medicines and screening the diverse plants and microbial resources of the country89. In terms of its size, diversity and access to talent and resources this programme is not only the world’s largest project of its kind, but is also unique96. Identification of antioxidant rich natural resources, preparing molecular fingerprints of their chemical compositions and studying the multiple therapeutic properties in this programme may help make India self-reliant in drug development in future.

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V. FREE RADICALS AND AGING

V. Free Radicals and Aging

The recent growth in knowledge of free radicals and reactive oxygen species (ROS) in biology is producing a medical revolution that promises a new age of health. In fact, the discovery of the role of free radicals in chronic degenerative diseases is as important as the discovery of the role of microorganism in infections disease (Bray, 1999).

It is well known that ROS is generating spontaneously in the living cell during several metabolic pathways. These comprise components of biological electron transport systems (photosynthetic, mitochondria, microsomal), including various enzymes and biomolecules: neutrophil, xanthine oxidase, cyclooxigenase, lipooxigenase, autooxidation of catechol amines (Halliwell and Gutteridge, 1986).

Although known as very harmfull, there is at least one case however, in which organism employ radicals in a controlled way to achieve a useful purpose: the action of phagocytic cell (neutrophils and monocytes, macrophage). Free radicals are necessary in the immune system, prostaglandine biosynthesys and antibacterial cell activities. Beside, above mentioned physiological systems, ROS generation are induced by several exogenous factors such are pollution, smoke, radiation, pesticide, drug consumption...

REACTIVE OXYGEN SPECIES

Highly reactive oxygen molecules and oxygen radicals are generated from the triplet state oxygen by excitation or reduction. Beside singlet state oxygen, having two higher and lower states $^1\Delta_g$ and $^1\Delta^+_g$, which arise mainly by photo excitation, more reactive reduced oxygen radicals are formed through univalent reduction of oxygen molecules. One electron reduction of oxygen produces superoxide $HO_2^{\cdot}$ Or $O_2^{\cdot -}$. The superoxide is degradaded into oxygen and hydrogen peroxide by disproportionation reaction.The hydrogenperoxide is fairly stable, but has week bonding energy and undergoes 1-electron reduction for form hydroxyl radical $OH^{\cdot}$ and water.

Why oxygen is toxic in biological systems?

SUPEROXYDE THEORY

Although for aerobic organism oxygen is necessary for life it has been accepted that oxygen is toxic. Explanation is in the “Superoxide theory of oxygen toxicity” which was postulated by Irwin Fridowich and Joe McCord in 1969 year, and states that oxygen is toxic because some of it is metabolized to make supeoxide radical. However it is not very reactive radical species, and it does not appear to react at

significant rates with DNA, phospholipids or proteins. Much of the toxicity of superoxide is thought to be due to its conversion into more damaging species, including peroxynitrite and hydroxyl radicals. Superoxide can react with some biomolecules. Its protonated form, $HO_2^{\cdot}$, is more reactive and can oxidize polyunsaturated fatty acids, although it has not been shown to be capable of attacking membrane lipids. Superoxide dismutation produces H_2O_2 that can exert some direct toxic effect and can be a precursor of $OH^{\cdot}$. Release of iron ions from iron-sulphur cluster (by $O_2^{\cdot -}$ or $ONOO^{\cdot}$) or from ferritin (by $O_2^{\cdot -}$) and from heam proteins by H_2O_2 can provide the iron needed for Fenton chemistry. Peroxynitrite is powerfull nitrating; nitrosylating and oxidizing species under physiological conditions and can also displace redox-active cupper from ceruloplasmin (Halliwell, 1999).

Although nor super-oxide radicals and nor H_2O_2 are highly reactive species, their activity as active oxygen species come from their potential to produce extremely highly reactive $HO^{\cdot}$ radicals through the Fenton reaction (I) and Haber-Weiss reaction (II):

$$H_2O_2 + Fe(II) \longrightarrow Fe(III) + OH^{\cdot} + OH^{-} \quad (I)$$

$$H_2O_2 + O_2^{\cdot -} \longrightarrow O_2 + OH^{\cdot} + OH^{-} \quad (II)$$

OH radical is because of its extreme reactivity the main factor of so-called oxygen toxicity. It reacts with all biological materials, oxidatively by hydrogen withdrawal, double bond addition, electron transfer and radical formation, and initiates autoxidation, polymerization and fragmentation.

LIPID PEROXIDATION

Is complex process occurring in aerobic cells and reflect the interaction between molecular oxygen and polyunsaturated fatty acids. This involves formation and propagation of lipid radicals ($L^{\cdot}$), uptake of oxygen, rearrangement of double bonds, generation of lipid alkoxyl ($LO^{\cdot}$), lipid peroxyl ($LOO^{\cdot}$) radicals, lipid hydroperoxide (LOOH) as well as variety of degradation products.

At least two paths are known for the formation of lipid peroxide *in vivo*. One occurs through autooxidation of catecholamine, thiols, quinones, and others, and redox reactions of oxyhemoglobin and myoglobin, and the other from active oxygen by the action of xanthine oxidase, NADPH oxidase, and other enzymes.

ROS AND LP IN HUMAN PATHOLOGY AND DISEASES

In the case of disturbed balance between formation of free radicals and antioxidant defense, in the cell we have oxidative stress and the free radicals can play a role in the development of various diseases.

Overproductions of ROS have been implicated in the etiology of host degenerative diseases including cardiovascular diseases, diabetes, cancer, Alzheimer's disease, retinal degeneration, ishemic dementa, and other neurovegetative disorders and aging. In addition they also play a role not only in acute conditions, such as trauma, stroke, and infection, but also in physical exercise and stress.

CARDIOVASCULAR DISEASES

Heart diseases continue to be the biggest killer, responsible for about half of all death in developed countries. Understanding and potentially controlling oxidative events as they affect cardiovascular disease (CVD) therefore, has the potential to provide enormous benefits to our population in health and lifespan.

Polyunsaturated fatty acids occur as a major part of the low-density lipoproteins (LDL) in blood and oxidation of these lipids components in LDL play a role in atherosclerosis. The three most important cell types in the vessel wall: endothelial cells, smooth muscle cell and macrophage can release free radical, which affect lipid peroxidation. With acontinuated high level of oxidized lipids, blood vessel damage to the reaction process continues and can lead to generation of foam cells and plaque the symptom of atherosclerosis. Oxidized LDL is atherogenic, and is thought to be important in the formation of atherosclerotic plaques. Furthermore oxidized LDL is cytotoxic and can directly damage endothelial cells (De Whallel et al., 1990).

CANCEROGENESIS

Numerous investigators have proposed participation of free radicals in carcinogenesis, mutation and transformation, particularly in the past 10 years. Although there is no definitive evidence that free radicals involvement is obligatory in these processes, it is clear that their presence in biosystem could lead to mutation, transformation and ultimately cancer (Simic, 1988). Induction of mutagenesis, the best known of the biological effect of radiation, occurs mainly through damage of DNA by the $HO^{\cdot}$ radical and other species produced by radiolysis of water, and also by direct radiation effect on DNA. The reaction of $HO^{\cdot}$ radicals are mainly addition to double bond of pyrimidine bases and abstraction of hydrogen from the sugar moiety resulting in chain scission of DNA. These effects can cause cell mutagenesis and carcinogenesis. Lipid peroxides are also suspected of being responsible for the activation of benzo(a)pyrene and other carcinogens, as well as for the production of some types of promoter.

Free Radicals and Aging

The human body is in constant battle to keep from aging. Strong experimental evidence supports the free radical theory of aging. An increasing number of diseases and disorder, as well as aging process itself, demonstrate link ether directly or indirectly to these reactive and potentially destructive molecules. Not much is known

about the mechanism of aging and what determine lifespan. Leading theories attribute these to programs written in DNA and/or to the accumulation of cellular and functional damage. Reduction of free radicals or decreasing their rate of production may delay aging and the onset of degenerative conditions associated with aging.

CO Q10 Prevents and Restores Heart Functions

Coenzyme Q10 has become one of the better-researched and substantiated "vitamin" supplements. Hundreds of studies document the multiple life-extension benefits of this versatile nutrient, not only as a powerful antioxidant, but also in augmenting the action of other antioxidants such as and in preventing such diseases as heart disease, neurological decline with age, and even periodontal disease.

Making Old Hearts Young Again

Coenzyme Q10: It may, indeed, be a time-reverser.

By Robert Van Kampler, M.D.

Heart attacks and other types of heart disease affect older people to a much greater extent than the young. Young hearts bounce back much better from stress and damage, even the stress of treatment itself. However, treatment with coenzyme Q10 is demonstrating its ability to radically improve the heart's ability to recover from disease and stress.

Scientists in Melbourne, Australia, are giving coenzyme Q10 to elderly people about to undergo cardiac surgery in a bid to make their old hearts young again. Dr. Franklin Rosenfeldt, head of cardiac surgical research at the Baker Institute, says he expects the treatment will make the hearts of people over the age of 70 perform as well as those of 30-year-olds.

Rosenfeldt believes CoQ10 will improve heart function in two ways. The antioxidant fights free radicals released at times of stress, such as during cardiac interventions (including angioplasty, thrombolysis, and surgery). It also improves the way cells convert oxygen and food to energy, strengthening the heart and making it beat more strongly.

People in their 70's and 80's are likely to be those who benefit most, and hence these are the first subjects of a current clinical trial. Rosenfeldt has already achieved good results in laboratory and animal trials.

"We are giving the patients CoQ10 for a week before surgery to build up the energy levels in their cells, and we are testing to see whether their recovery after surgery is better, whether their heart shows less damage, and whether cardiac tissue removed at

the time has greater energy capacity and also can stand up to stress better," Rosenfeldt says.

The double-blinded study, which began last June, is being conducted in two phases, a preliminary study involving 60 patients this year and the main study next year. (A double-blind study is one in which neither the subjects nor the persons administering the treatment knows which treatment a subject is receiving.)

Rosenfeldt says the results of cardiac treatments in elderly patients are known to be inferior to those in the young. In fact, the early mortality for elderly patients after such episodes as myocardial infarction, angioplasty, and cardiac surgery is up to three times greater than for younger patients. A possible reason is an age-related reduction in cellular energy transformation during the intervention, which may induce stress. Rosenfeldt expects to find that CoQ10 improves the response to this stress.

Several years ago he conducted a project in which he showed how aging rats respond to stress, and especially how their hearts respond. In treating both elderly (three years old, which is equivalent to an 80-year-old human) and young rats (six months old, equivalent to a 30-year-old human), Rosenfeldt demonstrated that young hearts recovered about 45% after stress, whereas elderly rats recovered only 18%. "There was a much poorer response to stress in elderly hearts," he noted.

In another test, conducted by Dr. Michael Rowland, Rosenfeldt and their colleagues, the rats were given CoQ10 or placebo for six weeks before the same tests were performed again. "In the senescent hearts," they noted, "pre-pacing cardiac work was 74% and oxygen consumption 66% of that in young hearts. CoQ10 was able to specifically protect the elderly hearts against stress. By comparison, the untreated senescent hearts showed reduced recovery compared with the young hearts. We concluded that senescent rat hearts have reduced baseline function and reduced tolerance to aerobic stress, compared with young hearts. By pre-treating the senescent hearts with CoQ10, the baseline function of the senescent myocardium and its tolerance to aerobic stress was greatly improved." This work has been accepted for publication in *Cardiovascular Research.*

That study was then repeated using human tissues. During open heart surgery, a small piece of tissue was removed from the heart to allow one of the tubes to be inserted for the heart/lung machine. Some of the tissue was tested in the laboratory, where it was put in an organ bath and allowed to contract in a fairly normal environment of oxygen to determine how much force it could generate. "We found we could have tissues from elderly patients or young patients and they all contracted quite well in the organ bath," Rosenfeldt notes.

In the next test, the tissue was subjected to stress in the form of ischemia (reduced blood flow), emulating the effects of a heart attack or cardiac surgery in the piece of tissue. This time, there was a large difference between recovery of the young tissue and elderly tissue, with the young tissue bouncing back by about 60%, but the older tissue recovering only about 40%.

However, when the tissues were incubated in the organ bath with CoQ10 and subjected to the same stress, the result was similar to that found in rats: the elderly tissues from patients aged more than 70 years recovered just as well as the young tissues. Rosenfeldt said CoQ10 has the potential to improve energy production in mitochondria by bypassing defective components in the respiratory chain, as well as by reducing the effects of oxidative stress. CoQ10 has emerged as a serious candidate for therapeutic use in the amelioration of bioenergetic defects manifested in the elderly heart.

In aged human atrial myocardium-the middle and thickest layer of the heart wall, composed of cardiac muscle-both hypoxia (reduction of oxygen) and simulated ischemia in vitro reveal a reduced capacity to recover pre-stress contractile function, compared with younger tissue. Rosenfeldt found that the frequency of mitochondrial dna deletion may be a useful molecular marker of stress-dependent, age-linked loss of tissue function. However, pre-treatment in vitro with CoQ10 overcomes the reduced capacity of senescent myocardium to recover contractile function after simulated ischemia, compared with younger tissue. (CoQ10 content is decreased in aged myocardium, and this decrease may play a role in the reduced post-stress recovery of contractile function.) Rosenfeldt presented this work at the inaugural meeting of the International Coenzyme Q10 Society in Boston last May.

In recent years, most of the clinical work with CoQ10 has centered on heart disease, mainly congestive heart failure but more recently as an adjunct to cardiac surgery. Congestive heart failure has been widely reported as being related to significantly low blood and tissue levels of CoQ10, and the severity of heart failure correlates with the severity of CoQ10 deficiency.

Several trials have compared the effect on heart function of giving CoQ10 or placebo, measured by echocardiography. The ejection fraction-the fraction of the blood pumped out of the heart with each beat-showed a gradual and sustained improvement with CoQ10. Moreover, patients reported a reduction in fatigue, difficult or labored breathing (dyspnea), chest pain, and palpitations. The most dramatic results were seen in patients who were started on CoQ10 soon after the onset of congestive heart failure, although those with more established disease also frequently showed clear improvement.
There have now been numerous studies in various countries detailing the use of coenzyme Q10 as a treatment in heart disease. The efficacy and safety of the

treatment has been well-established, including in large trials. One study, by Baggio et al., which took place in Italy, involved almost 2,664 patients with heart failure.
A study by Greenberg and Frishman found that 150 mg of CoQ10 reduced the frequency of angina attacks by up to 46%, while improving the capacity for physical activity in those patients. That work was published in the *Journal of Clinical Pharmacology* in 1990.

A study by Sunamori et al. published in 1991 reported that pre-treatment with coenzyme Q10 minimized the myocardial injury caused by cardiac bypass surgery and improved heart function, compared with patients not pre-treated with CoQ10 (*Cardiovascular Drugs and Therapy*, 5, 297-300). More recently, R.B. Singh, from the Heart Research Laboratory at the Medical Hospital and Research Center in Moradabad, India, told the inaugural conference of the International Coenzyme Q10 Association that, in a randomized double blind trial of 144 patients with acute myocardial infarction, coenzyme Q10 was seen to be associated with a significant reduction in angina pectoris, arrhythmias, and left ventricular dysfunction. Nonfatal infarction and cardiac deaths also were significantly lower in the coenzyme Q10 group than in the control group.

The future may be bright. At the conference, Dr. Peter Langsjoen noted that we are now at the beginning of an exciting new chapter in the clinical application of CoQ10 due to the rapid increase in public awareness and interest, all stimulating further clinical trials.

ANTIOXIDANT DEFENSE SYSTEM

Antioxidant defense system against oxidative stress is composed of several lines, and the antioxidants are classified into four categories based on function (Noguchi et al., 2000):

- First line of defense is the preventive antioxidants, which suppress formation of free radical (enzymes: glutathione peroxidase, catalase; selenoprotein, transferrin, ferritin, lactoferrin, carotenoids etc.)
- Second line of defense is the radical scavenging antioxidants suppressing chain initiation and/or breaking chain propagation reactions: radical scavenging antioxidants
- Third category: repair and de novo antioxidant (some proteolitic enzymes, repear enzymes of DNA etc)
- A fourth line is an adaptation where the signal for the production and reactions of free radicals induces formation and transport of the appropriate antioxidant to the right site.

Antioxidants act as: radical scavenger, hydrogen donors, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents.

Both enzymatic and non-enzymatic antioxidants exist in the intracellular and extracellular environmental to detoxify ROS. To provide maximum intracellular protection these scavengers are strategically compartmentalized thought the cell (table 1).

Table 1. Important enzymatic and non enzymatic physiological antioxidants.

Enzymatic antioxidants	**location**	**properties**
Superoxide dismutase (SOD)	Mitochondria, cytosol	Dismutase superoxide radicals
Glutathione peroxidase (GSH))	Mitochondria and cytosol	Removes hydrogen peroxide and organic hydroperoxide
Catalase (CAT)	Mitochondria and cytosol	Removes hydrogen peroxide
Nonenzymatic antioxidants	**location**	**properties**
Vitamin C	Aques phase of cell	Acts as free radical scavenger and recycles vitamin E
Vitamin E	Cell membrane	Major chain-breaking antioxidant in cell membrane
Uric acid	Product of purine metabolism	Scavenger of OH radicals
Glutathione	Nonprotein thiol in cell	Serves multiple roles in the cellular antioxidant defense
□-lipoic acid	Endogenous thiol	Effective in recycling vitamin C, may also be an effective glutathione substitute
carotenoids	Lipid soluble antioxidants, located in membrane tissue	Scavengers of reactive oxygen species, singlet oxygen quencher
bilirubin	Product of heme metabolism in blood	Extracellular antioxidant
ubiquinones	mitochondria	Reduced form are efficient antioxidants
Metals ions sequestration: transferrin, ferritin,		Chelating of metals ions, responsible for Fenton reactions

lactoferrin,		
Nitric oxide		Free radical scavenger, inhibitor of LP

NATURAL AND DIET-DERIVED ANTIOXIDANTS

Live forms living in the Earth's atmosphere must be equipped with systems to deal with the action of oxygen in living matter. Plants are especially susceptible to damage by active oxygen (exposed to radiation UV light) this is why plants developed numerous antioxidant defense systems that results in certain numbers of very potent antioxidants. Beside plants many of microbial and animal products as well as fermented products, seaweeds, protein hydrolisates were found to be powerful antioxidants. Daily foods contain a wide variety of free radicals scavenging molecules, thus vegetables, fruit, tea, wine are product rich in natural antioxidant compounds such. Among numerious antioxidants following plant secondary products are of particular interest (Larson, 1988):

1. Plant phenolics: phenylproponaoids, coumarines, flavonoids
2. polyphenolic: tannins, proanthocyanidins
3. nitrogen containing compounds; alkaloids, nonproteins aminoacids, isotiocyanate, indoles
4. phytosterols
5. carotenoids
6. chlorophyl derivatives

Plant phenolic, particularly flavonoids, tannins and phenylpropanoids are of particular interest. Much of the interest in the bioactivity of plant phenolic has been spurred by the dietary anomaly referred as the "*French paradox*" the apparent compatibility of a high fat diet with allow incidence of coronary atherosclerosis (Renaud&Lorgeril, 1992). Many so-called secondary products can act as potent bio-antimutagens. Cheng et al., (1989) showed antimutagen action of green tea extract, for which epigallocatecin gallate seems to be most responsible. Therefore, there is currently a strong interest in the study of natural compounds with free radical scavenger capacity and their role in human health and nutrition.

Dietary antioxidants may contribute to the decrease of cardiovascular disease by reduction of free radical formation as well as oxidative stress in general, by protection of LDL oxidation and platelet aggregation and by inhibiting synthesis of proinflamatory cytokines (Kushi, 1996). Epidemiological studies have shown that a higher intake of these compounds is associated with lower risk of mortality from cancer and coronary heart disease.

The use of spices has been valued from prehistorically times not soley because of their flavor, but also because of their food-preserving power. Numbers of studies have been done on their antioxidant activity as well as their antiseptic activity (Chipault et al., 1952; Madsen et al., 1997; Mimica-Dukic, 2001, Mimica-Dukic & Bozin, 2002, Bozin et al., 2002). Several compounds from spicy and aromatic plants are confirmed to posse’s strong antioxidant activity. Thus, phenolic diterpenoids: carnosol, rosmanol, carnosoic acid from sage (*Salvia officinalis* L.) and rosemary (*Rosmarinus officinalis* L.), in thyme (*Thymus vulgars* L.) dimers of thymol and flavonoids, flavonoids in oregano *(Origanum vulgare* L.) and pepper (*Piper nigrum* L.) were reported as strong antioxidative compounds. In our recent study we found that essential oils, as volatile compounds of many aromatic plants, besides their well known antimicrobial activity posses also significant antioxidant properties. As a strong free radical scavenger monoterpene ketones (thujone, menthone, carvone) and hydrocarbons are confirmed (Mimica-Dukic, 2001, Mimica-Dukic & Bozin, 2002, Bozin et al., 2002).

Therefore many aromatic and spicy plants as well as their essential oils, could serve not only as a flavor agent but also as a safe food antioxidant and supplement in preventing deterioration of foodstuff products. Consumption of food produced with natural essential oil or aromatic plant extracts are expecting to prevent the risk of many free radicals mediated diseases.

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