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Antioxidants: Their Role in Health and Disease

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Abstract: During the past few years there has been an escalation of interest in the role of antioxidants in health and disease. Antioxidants act as free radical scavengers and can thus play significant protective role in many age-related and chronic inflammatory diseases. This article reviews the role of antioxidants vitamin C, E and β -carotene in the process of ageing and diseases like coronary heart disease, Alzheimer's disease and cancer. The phenomenon of ageing has been mystery for mankind since long. Naturally occurring antioxidants can help slow this process by preventing us from harmful effects of ageing. The diet of healthy centenarians also shows high intake of antioxidants like vitamin A, C and E. On the other hand lipid peroxidation is necessary for atheroma formation in coronary arteries leading to heart attacks. This process begins early in childhood. By taking antioxidants this process can be significantly slowed down or even terminated. Reactive oxygen species may also lead to DNA changes and gene mutations, a primary pathology behind uncontrolled cell proliferation and tumorigenesis. Vitamin A and E reduce this stress and have been shown to decrease the risk of prostate and gastric cancers. Dementia and Alzheimer's affects a large proportion of elderly population. These are a result of oxidative damage going on in our brain from our early life. Antioxidants also contribute in preventing these neurodegenerative processes and may improve the standard of living of the elderly.

Key words: Anti-oxidants, vitamin A, C and E, coronary heart disease, Alzheimer's disease, cancer, ageing

INTRODUCTION

One of the most exciting areas of research nowadays is that of antioxidants including vitamin E and the provitamin A compound β -carotene. Other antioxidants include the watersoluble vitamin C and the synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene (BHT) and propyl gallate. Evidence strongly suggests that antioxidants known to protect foods from rancidity may also protect the body from harmful chemical reactions and possibly improve long-term health. Epidemiological data available suggests that high blood concentration or dietary intake of antioxidant vitamins may have a beneficial effect on many age related diseases, hence a forecast of an improvement in lifespan and an enhanced quality of life^[1]

The diet of the Greece prior to 1960, defined as the diet of the Crete, when analysed showed a high content of antioxidants (especially resveratrol and polyphenols) and other protective substances like selenium,

glutathione, vitamin E and C. These may well be the reason for their lower death rates and longer life expectancy. Hence the use of antioxidants is not new^[2]. To date the Recommended Daily Allowance (RDAs) for vitamins, including the antioxidants, are set to prevent nutritional deficiency diseases and maintain body stores. In a healthy body, pro-oxidants and antioxidants maintain a ratio and a shift in this ratio towards pro-oxidants gives rise to oxidative stress. This oxidative stress may be either mild or severe depending on the extent of shift and remains the cause of several diseases^[3]. In the present communication we consider the use of antioxidants to prevent the phenomenon of ageing, the development of cancers, Coronary Heart Disease (CHD), Alzheimer's disease and some other diseases, mainly of the chronic variety.

Dietary supplementation with β -carotene will not make you see better but may help you resist the development of CHD. However concerns have been raised that several vitamins may increase the rate of

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development of different cancers in high risk individuals, e.g., β -carotene^[1]. It therefore, needs to be emphasized that high doses of these antioxidants may prove harmful, even those that are essential nutrients. Such findings lead us to exercise some caution with nutrient supplementation. In spite of this there are those who would push for ingestion of levels of nutrients well above that found in typical well balanced diets.

Can oxygen be harmful: Antioxidants function chiefly to protect our food and us from the ravages of oxygen. How can this be? Oxygen is the most vital element in our environment; we can live for years without vitamin A in our diet but only about 4 to 5 min without oxygen. How can something so vital be so toxic, the answer in part lies in the fact that under the right conditions oxygen becomes much more active, forming Reactive Oxygen Species (ROS) called free radical species. These include single oxygen, hydrogen peroxide, superoxide anion radical and hydroxyl radical. Other major compounds include ozone and nitrogen-oxygen combinations that are important components of the air pollutants. The sources of these free radicals in living body are respiratory chain, phagocytes, Arachidonic Acid (AA) metabolism, cytophosphokinase, non-enzymatic reaction of oxygen and ionizing radiation. These compounds sometimes working together with some common metals like copper, iron and cobalt attack an important group of tissue constituents-notably lipids-creating a number of deleterious effects. Stress, anesthesia, anticancer drugs, pain killers and even normal energy metabolism also will release free radicals in varying amounts. These free radical reactions have been implicated in the pathogenesis of a growing number of disorders the free radical diseases^[4,5]. Evidence of free radical damage to DNA is commonly seen in urine as excretion of altered genetic material occurs simultaneously with DNA repair.

Fortunately, a considerable amount of protection exists in the body in the form of antioxidants and certain enzymes, like glutathione peroxidase, heme containing peroxidase, catalase and superoxide dismutase (SOD). These react directly with active oxygen or peroxides to reduce their reactivity and thereby help protect cells. Antioxidants like BHA, BHT and vitamin E supply an electron (actually hydrogen radical) to stabilize free radicals. Vitamin C helps to regenerate vitamin E. Still other antioxidants, chelators, tie up certain metals so that they cannot catalyze oxidation. With the entire foregoing protecting mechanisms available, do already healthy people need further protection in the form of supplementation?

Antioxidant enzymes: It appears that all mammalian cells contain endogenous antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase and catalase. Although the levels vary between cell types, they are very tightly controlled within all cells so as to maintain the redox balance. This is one of many homeostatic mechanisms observed in mammals and as with the others, very complex controls are involved, which remain to be fully elucidated.

In the early 1960s, a series of cellular enzymes was identified; these enzymes are able to detoxify free radicals by converting them back to more stable molecules within the cell, to be used or disposed of accordingly.

The first to be discovered and indeed the first in the chain of events, is an enzyme referred to as superoxide dismutase (SOD). SOD is responsible for the dismutation of oxygen radicals to hydrogen peroxides, which is subsequently detoxified by glutathione peroxidase. There are four isoforms of SOD. Copper/zinc SOD (Cu/Zn SOD) has two forms, one intracellularly and one extracellularly. Iron SOD (Fe SOD) is found in the cytosol of non-mammalian cells and manganese SOD (Mn SOD) functions solely in the mitochondria. It is this fourth isoform that is currently attracting a great deal of attention with regard to potential protection strategies, both for cancer and number of other high profile diseases, including ischaemic heart disease.

Antioxidants and ageing: Ageing is a universal phenomenon. It is the accumulation of changes responsible for the sequential alteration that accompany advancing age and the associated progressive increases in the chances of death. All individuals of a given species are dead by some age characteristic of the species. In spite of many biological and clinical observations on the changes that occur during ageing, understanding about the causes of ageing or the mechanisms determining the life span potential is being investigated. The ageing process is now considered to be the major cause of disease and death after about age 28^[6].

The free radical theory of ageing arose in 1954 from a consideration of the ageing phenomenon and the premise that a single common process modifiable by genetic and environmental factors was responsible for the ageing and death of all living things. The theory postulates that ageing is caused by free radical reactions, these reactions may be involved in the production of the age-related changes associated with the environment, disease and the intrinsic ageing process. Support for the free radical theory of ageing has increased progressively. It now includes: a) studies on the origin of life and evolution, b) studies on the effect of ionizing radiation on

living things, c) dietary manipulations of endogenous free radical reactions, d) the plausible explanations it provides for ageing phenomena and e) the growing numbers of studies that implicate free radical reactions in the pathogenesis of specific diseases^[7].

It is reasonable to expect on the basis of the present data, that the average life expectancy at birth can be increased by 5 or more years by nutritious low caloric diets supplemented with one or more free radical reaction inhibitors^[8,9]. However, many uncertainties remain, as the free radicals only occur in trace quantities in biological tissues, their cellular levels and actions cannot be measured in vivo and definitive proof that oxidized molecules are the primary cause of ageing is lacking. Moreover, ageing is also likely to be a multifactorial process and not reducible to any one single cause^[10].

A study done by Kasapoglu and Ozben^[11], investigated the correlation between the oxidative stress and ageing. They determined the levels of lipid peroxidation expressed as Thiobarbituric Acid Reactive Substances (TBARS) and Malonyl Dialdehyde (MDA), conjugated dienes; oxidative protein damage as indicated by carbonyl content and activities of antioxidant enzymes including Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPX) in a sample of 100 healthy men and women ranging in age from 20 to 70 years. In addition Vitamin C and E levels, reduced glutathione and sulphhydryl content were determined. The oxidation end product of nitric oxide (nitrate) was also studied to investigate any role of nitrogen radicals in ageing. The data suggested that the level of oxidative stress increase cannot entirely be attributed to a decrease in the activities of antioxidant defense system and probably various other factors are also contributing^[11].

Another study done by Mecocci *et al.*^[12] on healthy centenarians, to take a better look at the role of antioxidants in ageing, measured the plasma levels of vitamin C (ascorbic acid), uric acid, vitamin E (α -tocopherol), vitamin A (retinol), carotenoids, total thiol groups and the activity of plasma SOD and GPX as well as the activity of red blood cell. The results of the study made it evident that the healthy centenarians show a particular profile in which levels of vitamin A and E seem to be important in guaranteeing their extreme longevity.

Congy *et al.*^[13] determined the oxidative stress status and its significance in elderly subjects. Six parameters marking oxidative stress, namely TBARS, vitamin E, selenium, SOD, GPX were evaluated in 52 elderly patients (mean age 85+/-6 years; range 74 to 98). These were compared with those in 30 disease free young subjects (age range 20-40 years). The conclusion achieved was that, the parameters observed could be considered as a

biological marker of ageing and supplementation of selenium and other antioxidants was proposed.

Antioxidants and cardiovascular disease: Coronary artery disease is the major cause of death in developed countries^[14]. It may present as any of the three manifestations encompassed in the term Acute Coronary Syndrome. Despite the availability of good coronary care units and reduced in-hospital mortality rates, we see that about 75% of the MI patients are not even able to make it to the hospital. In 20% of the cases sudden death is the first and only manifestation^[15-17].

Oxidative stress is involved in the pathogenesis of atherosclerosis and antioxidants have been used in the clinical studies in the past few years for the prevention and treatment of atherosclerosis. A great deal of evidence exists that supports the idea that lipid peroxidation products are damaging to coronary arteries and that antioxidants help prevent such damage. It now appears that LDL must first undergo oxidation before plaque accumulations can occur. These modified LDL particles do not bind readily to the endogenous LDL receptor and are therefore not cleared from the circulation by this mechanism. They penetrate the arterial intima more easily, are more readily oxidized, possibly because they contain less antioxidant protection and are taken up by the macrophage scavenger receptors, accelerating foam cell formation. Because LDL is the primary carrier of fat-soluble vitamins it seems logical that antioxidant vitamin supplementation can fortify LDL with antioxidant power and increase resistance of LDL to oxidation. An approach to enhance the endogenous antioxidant defense system within the LDL particles is with lipophilic antioxidants such as α -tocopherol and β -carotene, or by supplementing the aqueous-phase antioxidant capacity with ascorbic acid^[18]. A double blind placebo controlled trial done by the Centre for Heart and Chest Research, Melbourne, Australia showed that the LDL Vitamin E content was increased by 127% after supplementation (1000 IU for three months) resulting in a significant reduction in the oxidative susceptibility of LDL^[19].

There are numerous studies also supporting these possibilities e.g. in a study, men and women who supplemented their diet for two or more years with at least 100 mg of Vitamin E per day had about 40% reduction in the risk of CAD^[20,21]. Another randomized controlled clinical trial performed in a total of 2002 patients, with angiographically proven CAD, Vitamin E supplementation (400 or 800 IU) prevented the combined end-point of non-fatal MI and cardiovascular death, with an overall risk reduction of 47%, but did not reduce mortality^[22]. An interesting point to raise here is that the increased role of

antioxidants in the patients with hypercholesterolemia and chronic smoking, long term Vitamin E supplementation improves endothelial dependent relaxation in forearm resistance vessels of chronic hypercholesterolemic smokers^[23].

Several major randomized trials of antioxidant supplementation for the primary and secondary prevention of cardiovascular disease are currently in progress. The Women's Health Study (WHS), the Women's Antioxidant Cardiovascular Disease Trial (WACDT) in the USA and the Heart Protection Study Oxford, UK are underway and hopefully will provide reliable data to define the role of antioxidants as primary and secondary preventive measures for cardiovascular disease.

One key aspect of antioxidants virtually ignored by the medical professionals is the function of platelets. These take part in blood clots that trigger the heart attack by lodging in arteries partially blocked by plaque. Again, antioxidants to the rescue! Vitamin E reduces the tendency of platelets for forming clots^[24].

Antioxidants and inflammatory diseases: Inflammation is characterized by a respiratory burst of activated neutrophils and macrophages, leading to the destruction of invading micro-organisms. The mechanism is a useful function protecting against attack, however, the inflammatory response can also be detrimental as it is non-specific and may lead to the development of inflammatory diseases such as rheumatoid arthritis, Systemic Lupus Erythematosus (SLE), inflammatory bowel disease and psoriasis.

There is also a strong relationship between Reactive Oxygen Species (ROS), Free radicals and Inflammatory diseases. Free radicals are thought to act indirectly as cellular messengers and elicit an inflammatory response. Over-production of these species may cause oxidative modification of biological molecules e.g. trypsin, collagen, LDL, DNA and lipids.

ROS and free radicals also activate a series of enzyme systems including protein kinases, protein phosphatases, transcription factors and heat shock proteins. ROS are also critical for gene expression which encode inflammatory proteins e.g. proteinases involved in tissue destruction such as collagenases and gelatinases. Nuclear factor- κ B (NF- κ B) has been implicated in AIDS, as HIV is NF- κ B dependent. In the case of rheumatoid arthritis, rheumatoid factor binds IgG when it is exposed to free radicals. This binding stimulates the production of more free radicals which then attack the cartilage matrix.

Furthermore, there is evidence that antioxidant supplementation can alleviate inflammation. A study of

vitamin E in the synovial fluid of patients suffering from rheumatoid arthritis found significantly lower levels than those measured in healthy individuals^[25]. Another study found low levels of vitamin E, β -carotene and selenium to be associated with increased risk of rheumatoid arthritis^[26]. The importance of iron in rheumatoid disease has also been reported^[27].

It has also been reported that the level of antioxidants drops dramatically in the presence of severe intestinal inflammation (inflammatory bowel diseases—ulcerative colitis and Crohn's diseases are chronic gastrointestinal inflammatory diseases of unknown etiology. Decreased oral intake, malabsorption, accelerated nutrient losses and drug nutrient interactions cause nutritional and functional deficiencies that require proper correction by nutritional therapy, Glutamine, short chain fatty acids, antioxidants and omega 3 fatty acids are an important therapeutic alternatives in the management of Inflammatory Bowel Disease. Evidence of the protective effects of higher consumption of olive oil, fruit vegetables and β -cryptoxanthin.

Supplements with long chain n-3 (PUFA) consistently demonstrates an improvement in the symptoms associated with inflammation. Antioxidants, zinc, iron, folate and other vitamins, calcium, Vitamin D and fluoride are also recommended. The RA patients should also consume a balanced diet rich in long chain n-3 PUFA and antioxidants.

Antioxidants and cancer: Over the past three decades, extensive efforts were made to treat cancer. However, as recent statistics show, the incidence of and mortality from cancer have in general not diminished but have instead increased^[28,29]. Epidemiologic and laboratory studies indicate that a high consumption of antioxidant-rich fruit and vegetables can reduce the risk of cancer^[30,31]. ROS are essential for various cell defense mechanisms; they can also cause oxidative damage to DNA, proteins and lipids, resulting in potentially enhanced risk of cancer. Several antioxidants like vitamin C, E and β -carotene can help maintain appropriate balance between the desirable and undesirable cellular effects of ROS.

Vitamin C is considered to be one of the most prevalent antioxidant components of fruits and vegetables and it could exert chemopreventive effects without apparent toxicity at doses higher than the current RDA of 60 mg dL⁻¹^[32,33]. Henson *et al.*^[34] analyzed 46 epidemiologic studies on the protective effects of vitamin C against various types of cancers; 33 of these studies found a significant link between vitamin C intake and a reduced incidence of cancer. A more recent analysis by Carr *et al.*^[35] showed that vitamin C acts as an

antioxidant *in vivo*. Of the 44 published *in vivo* studies examined, 38 reported a decrease in the number of markers of oxidative damage to DNA, lipid, or protein; 14 showed no changes and only 6 reported an increase in oxidative damage after supplementation with vitamin C. Drake *et al.*^[36] studied 82 patients with helicobacter associated gastritis, a condition which clearly predisposes to gastric cancer and demonstrated increased vitamin C associated free radical scavenging activity which otherwise could lead to DNA mutation and carcinogenic changes in gastric mucosa. In 1997, expert panels at the world cancer research fund and the American Institute for Cancer Research estimated that vitamin C can reduce the risk of stomach, mouth, pharynx, esophagus, lung, pancreas and cervical cancers by 30%. Vitamin C appears to decrease oxidative stress there by preventing DNA damage, which is implicated in tumor initiation. On the other hand, recent studies showing that it has protective effects on gap junction intercellular communication by inhibiting the production of hydrogen peroxide. Inhibition of cell-cell communication is strongly correlated with carcinogenesis. Bowie and O'Neill^[37] demonstrated that vitamin C inhibits expression of transcription factor NF- κ β through p38 mediated mitogen protein kinase. Altered expression of NF- κ β has been implicated in pathogenesis of several chronic diseases including cancer. Furthermore, Lutsenko *et al.*^[38] recently showed that vitamin C can prevent hydrogen peroxide induced mutations in human cells which are inadvertently linked to tumor initiation and tumorigenesis. Therefore, apart from antioxidant activity of vitamin C, it acts through several intracellular targets to act as a chemopreventive agent.

Vitamin E's role to prevent carcinogenesis has been a topic of much interest due to its strong antioxidant properties. Large interventional trials have proved its chemoprotective role in case of prostate cancer. Albanes *et al.*^[39] showed in the α -Tocopherol and β -carotene (ATBC) study, male smokers who took vitamin E supplements had a 34% lower incidence of prostate cancer and 41% lower mortality from prostate cancer than did those who did not take the supplements^[40]. In a study in Linxian, China, approximately 15 000 of 29 584 Chinese adults received a mixture of 30 mg α -tocopherol per day, 50 mg selenium yeast/d and 15 mg β -carotene/d for 5.5 year. The subjects who received this mixture had a 13% lower incidence of cancer and a 10% lower mortality from stomach and oesophageal cancer than did the subjects who did not receive the mixture^[41]. But there are pros and cons to the use of antioxidants. Several studies have shown prooxidant effect of many naturally occurring antioxidants like vitamin C and selenium at different doses^[42].

Antioxidants and alzheimers disease: Alzheimers Disease (AD) is characterized by regional neuronal degeneration, synaptic loss and the presence of neurofibrillary tangles and senile plaques^[43]. AD has become the most leading cause of dementia in several developed and underdeveloped countries. It has the potential to become the most overwhelming public health concern of this century owing to increasing life expectancy and growth in the ageing population^[44].

A decade of research has suggested that ROS may contribute to the neuronal damage in AD. *In vitro* studies have reported a strong correlation between A- β protein and generation of free radicals^[45,46]. The increased production and deposition of A- β protein are early events in the pathogenesis of AD, that precede other changes such as the formation of tau, amyloid production and deposition, may be associated with increased oxidative stress. Hence the presence of antioxidants may provide protection to neurons and preserve cognitive function.

Several studies have suggested the use of antioxidants and the reduced risk of Alzheimer's disease. In the Cache county study by Zandi *et al.*^[47], 4740 elderly patients were prospectively analyzed and intake of vitamin E and C were correlated with decreased risk of AD. In other study in 2000, a placebo-controlled trial on moderately advanced AD patients was conducted and subjects were given 2000 IU of vitamin E daily. The results indicated the use of vitamin E with slow functional deterioration of AD patients^[48]. Charlton *et al.*^[49] and Helmer *et al.*^[50] have shown the decrease plasma levels of vitamin C and E are correlated with the risk of Alzheimer's disease. Charlton *et al.*^[49] conducted a case control study on 78 subjects and found that plasma levels of vitamin C were significantly less in cases as compared to controls. Dietary intake of vitamin C was same in both the groups. Their result thus adds to the theory of oxidative neuronal damage in AD, therefore, it can be argued if antioxidant supplement in people at risk of developing AD is increased, neuronal damage can be prevented. Helmer *et al.*^[50] conducted a case control study on French population. Their results clearly demonstrated that subjects with decreased plasma concentration of vitamin E are at increased risk of developing AD. Furthermore Craft *et al.*^[51] have recently shown the presence of increased amount of tocopherol, β -carotene and ascorbic in frontal lobe as compared to other lobes of brain^[51]. Frontal lobe is generally most vulnerable in Alzheimer's disease. The results also showed age related decrease in tocopherol and carotenoids in frontal lobe.

Engelhart *et al.*^[52] assessed the effects of antioxidants on cognitive function^[52]. The study design was based in

Netherlands and was conducted on 5395 men and women who were at least 55 years of age and were participants in Rotterdam Study and were free of dementia at baseline. After a 6 year follow up, they found reduced risk of AD in subjects taking dietary supplements of vitamin C and E. This effect was specially pronounced in smokers. However, the results of several studies have not been convincing and they suggested a rather meager role of intake of antioxidants in order to prevent AD^[53]. Morris *et al.*^[54] came up with a very interesting finding while investigating the role of antioxidants in the prevention of AD. They found out that vitamin E from food prevents AD but only in subjects who have APOE epsilon 4 allele absent. Several animal model studies have also come with the same conclusion. These types of studies could possibly explain the result of several other studies which have found no relationship with the use of antioxidants and AD.

The debate goes on and the presence of allelic polymorphism in beneficent subjects makes it even more interesting. Well-designed intervention trials, as well as observational investigations based on longer cohorts, with longer study duration and using multiple methodologies to assess molecular and antioxidants effects are necessary to clearly define the role of antioxidants in AD.

CONCLUSIONS

Overall, the risks of antioxidant supplementation are virtually nonexistent if some knowledge and common sense are applied. Vitamin E toxicity has been noted in rats but at a level equivalent to a 100 kg (220 pound) man taking 13 pounds of pure vitamin E in a month. Likewise, people taking extremely high levels of vitamin C and β -carotene are at a risk of various disagreeable effects.

Conditions that might suggest the use of supplemental antioxidants include smoking, living in a smoggy environment a familial risk for CHD and cancer. Since life itself is associated with free-radical production, everyone could benefit. We propose a general recommendation that is simple, inexpensive and reasonable. First, be sure to eat a lot of fruits, vegetables and whole-grain foods. Second, 0.5-1.0 g of vitamin C, 100-200 mg of vitamin E and 20 mg of β -carotene per day should offer additional protection. Smokers may need more.

By far the majority of us seem able to live healthy lives by eating a variety of foods that provide more than ample quantities of nutrients. Because of the examples of such uncommon but justified needs for supplement use, the lack of detailed knowledge that any given individual has for his or her specific nutrient

needs and the misunderstanding about RDAs and similar population-based recommendations, there is impetus for those with an insecurity borne of uncertainty to take supplements.

Several unfortunate cases of self-prescribed diet therapy should warn us of possible dangers. For example, administration of high levels of vitamin E can exacerbate blood coagulation defects in vitamin K-deficient individuals, including those who are given a vitamin K antagonist as medication to decrease chances for clots and ongoing risk of cardiovascular events. High amounts of vitamin C pose a risk for those who tend to over store iron (hemochromatosis). Vitamin C can enhance the absorption of iron, as well as inhibit copper absorption.

From, all of the above it would seem more prudent to recognize we must learn more before any scientifically secure recommendation can be made about what, if any, supplementation is useful. Until then, most of us would be better served if we abide by the proven recommendation of obtaining our nutrients by eating in moderation a variety of healthy food.

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