

Review

Free radicals and antioxidants in health and disease

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Introduction

It is ironic that oxygen, an element indispensable for life can, under certain situations, have severely deleterious effects on the human body. Most of the potentially harmful effects of oxygen are due to the formation and activity of a number of chemical compounds, known as reactive oxygen species, which have a tendency to donate oxygen to other substances. Many such reactive species are free radicals and have a surplus of one or more free-floating electrons rather than having matched pairs and are, therefore, unstable and highly reactive. Types of free radicals include the hydroxyl radical (OH[•]), the superoxide radical (O₂^{•-}), the nitric oxide radical (NO[•]) and the lipid peroxy radical (LOO[•]).

Production of free radicals in the human body

Free radicals and other reactive oxygen species are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants and industrial chemicals.

Free radical formation occurs continuously in the cells as a consequence of both enzymatic and non-enzymatic reactions (Figure 1). Enzymatic reactions which

serve as sources of free radicals include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis and in the cytochrome P450 system. Free radicals also arise in non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations. Some internally generated sources of free radicals are [1]:

- mitochondria
- phagocytes
- xanthine oxidase
- reactions involving iron and other transition metals
- arachidonate pathways
- peroxisomes
- exercise
- inflammation
- ischaemia/reperfusion.

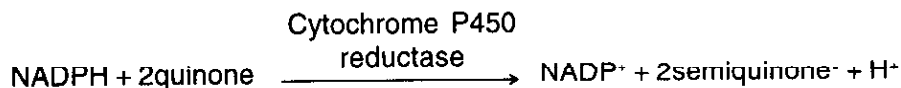
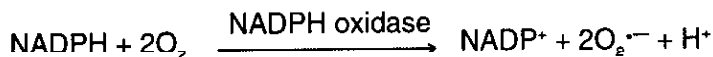
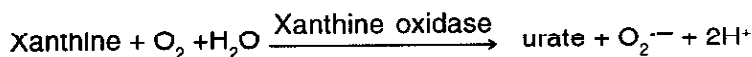
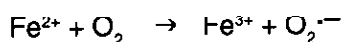
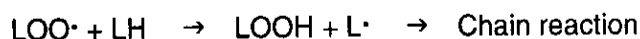
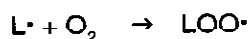
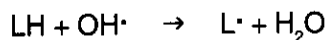
Some externally generated sources of free radicals are [1]:

- cigarette smoke
- environmental pollutants
- radiation
- ultraviolet light
- certain drugs, pesticides, anaesthetics and industrial solvents
- ozone.

With electrons unhinged, free radicals roam the body, wreaking havoc. The free radical, in an effort to achieve stability, at-

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Enzymatic free radical formation**Nonenzymatic free radical formation****Lipid oxidation by radical attack (L = lipid)****Figure 1 Examples of free radical reactions**

tacks nearby molecules to obtain another electron and, in doing so, damages those molecules. This situation can be compared to letting a bachelor into a dance where people have come as couples. The bachelor begins cutting in, each time leaving another bachelor, so the breaking up of couples spreads through the dance floor.

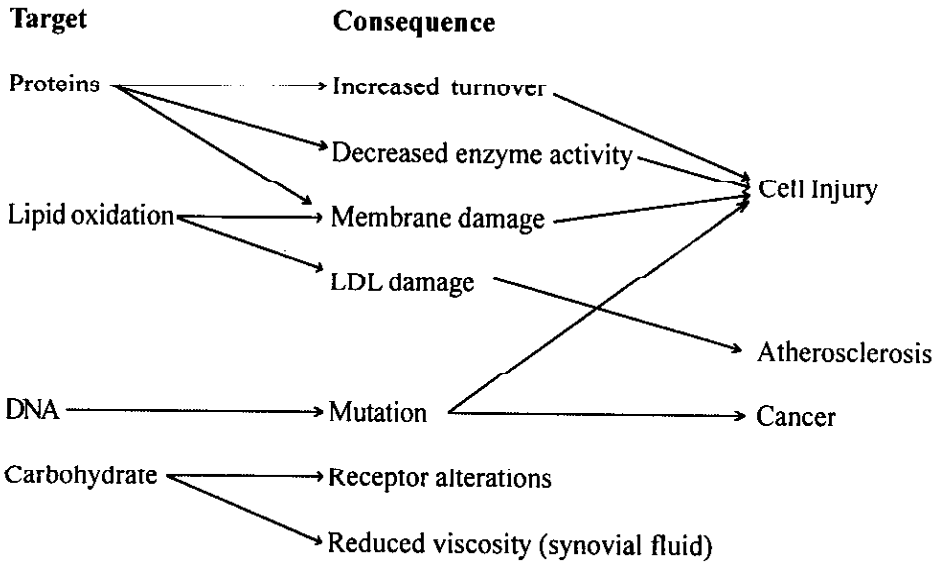
If free radicals are not inactivated, their chemical reactivity can damage all cellular macromolecules including proteins, carbohydrates, lipids and nucleic acids (Figure 2). Their destructive effects on proteins may play a role in the causation of cataracts. Free radical damage to DNA is also implicated in the causation of cancer and its effect on LDL cholesterol is very likely responsible for heart disease. In fact, the theory associating free radicals with the aging

process has also gained widespread acceptance (see Box 1).

The free radical diseases

A well accepted fact is the increasing incidence of disease with advancing age. A plausible explanation for the association of age and disease is based on the implication of free radical reactions in the pathogenesis of several disorders.

Free radical reactions are expected to produce progressive adverse changes that accumulate with age throughout the body. Such "normal" changes with age are relatively common to all. However, superimposed on this common pattern are patterns influenced by genetics and environmental



Adapted from reference [1]

Figure 2 Free radical damage

differences that modulate free radical damage. These are manifested as diseases at certain ages determined by genetic and environmental factors. Cancer and atherosclerosis, two major causes of death, are salient "free radical" diseases. Cancer initiation and promotion is associated with chromosomal defects and oncogene activation. It is possible that endogenous free radical reactions, like those initiated by ionizing radiation, may result in tumour formation. The highly significant correlation between consumption of fats and oils and death rates from leukaemia and malignant neoplasia of the breast, ovaries and rectum among persons over 55 years may be a reflection of greater lipid peroxidation [2]. Studies on atherosclerosis reveal the probability that the disease may be due to free radical reactions involving diet-derived lipids in the arterial wall and serum to yield peroxides and other substances. These compounds in-

duce endothelial cell injury and produce changes in the arterial walls [3].

Free radicals, however, are not always harmful. They also serve useful purposes in the human body. Several observations indicate that the oxygen radicals in living systems are probably necessary compounds in the maturation processes of cellular structures. Furthermore, white blood cells release free radicals to destroy invading pathogenic microbes as part of the body's defence mechanism against disease. Hence, the complete elimination of these radicals would not only be impossible, but also harmful.

Counteracting free radical damage

The human body has several mechanisms to counteract damage by free radicals and

Box 1 Some clinical conditions in which free radicals are involved**Brain and nervous system*

Parkinson disease
 Actions of neurotoxins
 Vitamin E deficiency
 Hypertensive cerebrovascular injury
 Allergic encephalomyelitis
 (demyelinating diseases)
 Potentiation of traumatic injury

Eye

Cataract
 Age-related macular degeneration
 Photic retinopathy
 Ocular haemorrhage
 Retinopathy of prematurity

Heart and cardiovascular system

Atherosclerosis
 Keshan disease (selenium deficiency)
 Alcohol cardiomyopathy

Kidney

Autoimmune nephrotic syndromes
Reproductive functions
 Sperm abnormalities
 Germ-line mutations leading to congenital malformations
 Childhood cancer
 Hypertensive complications of pregnancy

Gastrointestinal tract

Nonsteroid-anti-inflammatory-drug-induced GI tract lesions
 Oral iron poisoning
 Endotoxin liver injury
 Liver injury
 Free-fatty-acid-induced pancreatitis

Lung

Hypoxia
 Cigarette smoke effect
 Emphysema
 Adult respiratory distress syndrome
 (some forms)
 Effects of oxidant pollutants (ozone, SO₂, NO₂)

Red blood cells

Faltoni anaemia
 Sickle cell anaemia
 Favism
 Malaria
 Protoporphyrin photo-oxidation

Cancer

Alcohol-related diseases

*Aging**Radiation injury*

Inflammatory disorders/immune function
 Age-related decline in immune function
 Autoimmune diseases
 Rheumatoid arthritis
 Glomerulonephritis
 Vasculitis (hepatitis B virus)

Leprosy

Iron overload
 Nutritional deficiencies
 Dietary iron overload
 Idiopathic haemochromatosis

Ischaemia/reperfusion

Stroke/myocardial infarction
 Organ transplantation

Exercise-induced oxidative stress

* Adapted from reference [1]

other reactive oxygen species. These act on different oxidants as well as in different cellular compartments.

One important line of defence is a system of enzymes, including glutathione peroxidases, superoxide dismutases and catalase, which decrease concentrations of

the most harmful oxidants in the tissues. Several essential minerals including selenium, copper, manganese and zinc are necessary for the formation or activity of these enzymes. Hence, if the nutritional supply of these minerals is inadequate, enzymatic de-

fences against free radicals may be impaired.

The second line of defence against free radical damage is the presence of antioxidants. An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. Some such antioxidants, including glutathione, ubiquinol and uric acid, are produced during normal metabolism in the body. Other lighter antioxidants are found in the diet. Although about 4000 antioxidants have been identified, the best known are vitamin E, vitamin C and the carotenoids. Many other non-nutrient food substances, generally phenolic or polyphenolic compounds, display antioxidant properties and, thus, may be important for health.

Although a wide variety of antioxidants in foods contribute to disease prevention, the bulk of research has focused on three antioxidants which are essential nutrients or precursors of nutrients. These are vitamin E, vitamin C and the carotenoids. Each of these antioxidant nutrients have specific activities and they often work synergistically to enhance the overall antioxidant capability of the body (Table 1).

The balance between the production of free radicals and the antioxidant defences in the body has important health implications. If there are too many free radicals produced and too few antioxidants, a condition of "oxidative stress" develops which may cause chronic damage. As mentioned above, free radicals have been implicated in several health problems. Cancer, atherosclerosis, cerebrovascular accidents, myocardial infarction, senile cataracts, acute respiratory distress syndrome and rheumatoid arthritis are just a few examples. Numerous studies have shown the protective effects of antioxidant nutrients on these health problems.

Table 1 Antioxidant functions of vitamins E, and C and β -carotene

Nutrient	Function
Vitamin E	Chain breaking antioxidant Free radical scavenger Singlet oxygen quencher
Vitamin C	Free radical scavenger Singlet oxygen quencher Regeneration of vitamin E
β -carotene	Singlet oxygen quencher Chain breaking antioxidant Free radical scavenger

Adapted from reference [4]

Vitamin E as an important antioxidant

Vitamin E is the collective name for eight compounds, four tocopherols and four tocotrienols, found in nature. It is a fat-soluble substance present in all cellular membranes and is mainly stored in adipose tissue, the liver and muscle. Vitamin E is a principal antioxidant in the body and protects polyunsaturated fatty acids in cell membranes from peroxidation. It is a singlet oxygen quencher, neutralizing these highly reactive and unstable singlet oxygen molecules. In fact, singlet oxygen can damage DNA and be mutagenic. Vitamin E also protects the double bonds of β -carotene from oxidation and thus exhibits a sparing effect. Due to the ability of vitamin E to work at higher oxygen pressures, free radicals are scavenged and tissue injury is minimized. Besides its anti-aging properties, vitamin E is known to afford protection against cancer, ischaemia and reperfusion injury, cataract, arthritis and certain neurological disorders.

Vitamin E and cancer

Cancer is the culmination of a multistep process that occurs over a period of several years or decades. The underlying cause is thought to be DNA damage, much of which is oxidative in nature. These oxidative processes, the mechanisms of which are not clearly understood, occur during the promotional stage of carcinogenesis. Hence, antioxidants may be able to cause regression of premalignant lesions or inhibit their development into cancer.

Several functions of vitamin E are relevant in considering its role in cancer prevention and control. Besides being a free radical scavenger, vitamin E at high intakes enhances the body's immune responses. Vitamin E also inhibits the conversion of nitrites in the stomach to nitrosamines, which are cancer promoters. A large multicentric case control study conducted by the US National Cancer Institute associated the use of vitamin E supplements with a 50% reduction in oral cancer risk, while dietary vitamin E and multi-vitamins had no significant effect [1]. However, another study demonstrated the inverse relationships of both dietary vitamin E and vitamin E supplements with breast cancer risk [5]. Hence, further trials are necessary to substantiate the role of vitamin E in cancer prevention.

Vitamin E and cardiovascular disease

Oxidative stress may affect cardiovascular function in two ways; one involving the long-term development of atherosclerosis and the other involving immediate damage during a heart attack or stroke. Free radicals may contribute to atherogenesis by oxidizing low density lipoproteins (LDLs) which then damage arterial walls. The oxidation of LDL cholesterol is suspected to occur at the initial stages of atherosclerosis, and vitamin E

has been shown to inhibit this oxidative reaction.

During an ischaemic episode, the supply of blood and oxygen to the tissues is cut off and consequently cells begin to die. Restoration of blood flow and oxygen supply by reperfusion can prevent tissue damage. However, harmful free radicals are generated during reperfusion and can damage tissues further. Vitamin E intakes are associated with lowered risk of angina and mortality from ischaemic heart disease.

Another significant factor in the development of atherosclerosis and other vascular diseases is platelet hyperaggregability. Vitamin E is known to inhibit platelet aggregation, as well as prostaglandin synthesis which stimulates platelet aggregation. Epidemiological evidence strongly links high vitamin E intake to reduced risk of coronary heart disease. Stephens et al. demonstrated that vitamin E treatment also significantly reduced the risk of cardiovascular deaths as well as non-fatal myocardial infarctions [6].

The protective effects of vitamin E, however, have been witnessed at high doses, much more than can be consumed through the normal diet. Whether vitamin E levels in normal diets are protective against heart disease still remains to be verified. Further research is needed to confirm the role of vitamin E, as well as to determine the optimal intake to protect against cardiovascular risk.

Vitamin E and eye disorders

Oxidative processes have been implicated in the causation of both cataracts and the age-related disorder of the retina, maculopathy. Cataracts occur when the transparent material in the lens of the eye becomes cloudy and opaque. Oxidation, induced mainly by exposure to ultraviolet light, is believed to be a major cause of damage to the proteins of the lens. The oxidized pro-

tein precipitates out and causes cloudiness of the lens. Antioxidants and antioxidant enzymes inactivate harmful free radicals and the protein-splitting enzymes (proteases) remove the damaged portion from the lens, but the oxidative damage occurs at a faster rate. Hence, oxidized protein may accumulate, and with time, the damage becomes irreversible.

All three of the major dietary antioxidants (vitamin C, vitamin E and carotenoids) have been associated with decreased cataract risk through the retardation of lens opacity. These findings have been supported by several epidemiological studies conducted in various parts of the world. Results of such studies of nutrients and cataract, however, have not been consistent. Some studies have shown protective effects of antioxidants against some types of cataract, but others have not [7]. Rouhiainen et al., for example, reported that low plasma levels of vitamin E were associated with the worsening of early cortical lens opacities [8].

Some scientific evidence suggests that excessive exposure to light, particularly blue light, is associated with age-related macular degeneration. While vitamin E has thus far not been associated with maculopathy, carotenoids are said to decrease the risk of this disorder.

Vitamin E and neurological disorders

The role of antioxidants in slowing the progression of certain neurological disorders has been suggested as oxidation may be a causative factor in several disorders of the nervous system. Supplementation with vitamin C and E might be of benefit in slowing the progression of Parkinson's disease. Further trials, however, need to be conducted to substantiate these claims.

Vitamin E and aging

Cellular damage by active oxygen species, including damage associated with lipid peroxidation, contributes to the pathological changes attributed to aging. Buttner and Burns reported a decrease in the rate of free radical mediated lipid peroxidation on supplementation with vitamin E [9]. Improvement in mental well-being of the elderly has been reported with vitamin E and selenium supplements. Vitamin E is also associated with improvements in arthritis.

Vitamin C

Vitamin C, or ascorbic acid, is a water-soluble vitamin. This vitamin is a free radical scavenger and interacts with free-radicals in the water compartment of cells as well as in the fluids between cells. It is considered to be one of the most important antioxidants in extra cellular fluids. Vitamin C has a sparing effect on vitamin E as it regenerates vitamin E from the tocopheroxyl radical after it has neutralized free radicals. Its protective effects extend to cancer, coronary artery disease, arthritis and aging.

Vitamin C and cancer

Vitamin C is effective in protecting tissues against oxidative damage. It suppresses the formation of carcinogens such as nitrosamines and quinones. Numerous studies have reported the protective effect of fruit and vegetable consumption on incidence of cancer [10]. This is mainly attributed to the protective effect of vitamin C against cancer.

Vitamin C and cardiovascular disease

A major culprit in the development of atherosclerosis is oxidized LDL. Vitamin C protects against this oxidation. There is

also evidence that vitamin C increases HDL levels and may also lower total cholesterol in the blood, thus reducing the risk of cardiovascular disease. While certain studies have failed to show any association between vitamin C supplementation and coronary risk, studies among various European populations indicate that coronary heart disease mortality is higher in those with blood vitamin C levels that are near or in the deficient range.

Several epidemiological studies note an inverse association with vitamin C status and blood pressure. However, further research is needed to substantiate these claims which presently appear inconsistent.

Vitamin C and cataracts

Use of vitamin C supplements has been inversely associated with cataract risk. High intake of fruits and vegetables which are rich sources of ascorbic acid appear to be protective too. In several epidemiological studies, cataract patients were shown to have low vitamin C and E intakes and low plasma vitamin C levels. Smokers are known to have lower blood levels of vitamin C than non-smokers and, as smoking induces free radical formation, they are at increased risk of developing cataracts.

Vitamin C and other diseases

Since oxidative processes are implicated in a variety of clinical disorders, antioxidants like vitamin C may play a significant role in their risk reduction. Supplementation with vitamin C and E, for example, has been reported to prove beneficial in Parkinson disease. Cigarette smoking is associated not only with reduced sperm count and poor sperm quality but also with lower blood vitamin C levels. Supplementation with vitamin C has been shown to improve sperm quality in heavy smokers. It has also been reported to reduce oxidative damage to

sperm DNA. Further research, however, needs to be undertaken to examine the role of such antioxidants in reducing infertility in men who are heavy smokers or who are exposed to oxidative stress from other causes.

Carotenoids

Carotenoids are a group of red, orange and yellow pigments found in plant foods, particularly fruits and vegetables. Some carotenoids like β -carotene act as a precursor of vitamin A; others do not. β -carotene is an effective antioxidant as it is one of the most powerful singlet oxygen quenchers. It can dissipate the energy of singlet oxygen, thus preventing this active molecule from generating free radicals. Its other antioxidant properties include the scavenging of free radicals. Unlike other nutrient antioxidants, β -carotene is efficient at low oxygen pressure. Besides β -carotene, other important dietary carotenoids include α -carotene, lycopene, lutein, zeaxanthin and β -cryptoxanthin. Recent studies have confirmed that lycopene has a powerful role as an antioxidant.

Carotenoids and cancer

The results of recent epidemiological studies have suggested that several carotenoids may be associated with reduced cancer risk. The evidence is much stronger in the case of β -carotene. Numerous studies have reported reduced risk of certain cancers with a high dietary intake of β -carotene. β -carotene may alter the way cancer cells communicate with normal cells. Cancer cells multiply rapidly when growth regulating signals from adjoining normal cells cease. Carotenoids act as "arbitrators" by enhancing the communication between the two cells and helping the cancer cells to act

more like normal cells. Furthermore, if cancer cells receive the growth regulating signals, tumour growth may be suppressed.

Several clinical trials showed regression in precancerous lesions of the cervix and the lung as well as the oral cavity with the administration of β -carotene. Studies by Zheng et al. [11] and Negri et al. [12] have reported that dietary antioxidants, especially β -carotene, may be important in the prevention of stomach and breast cancer. Whether the protective effects are attributable to the antioxidant nutrients themselves or to other substances found in the same foods, however, needs to be verified.

A few contradictory reports have also emerged. These reveal that β -carotene supplementation does not lower the rate of lung cancer [13,14,15]. β -carotene also does not reduce recurrence of adenomatous polyps and skin cancer [16,17]. More definite data need to be generated in the next few years for a clearer picture to emerge.

β -carotene and cardiovascular disease

The current evidence linking carotenoids with heart disease prevention is limited but promising. The European Community Multicentric Study on Antioxidants, Myocardial Infarction and Breast Cancer has shown that low β -carotene concentrations in adipose tissue were associated with a high risk of myocardial infarction in current smokers [18].

Clinical trials conducted by Hennekens et al. on US male physicians showed no significant effects, positive or negative, on cardiovascular diseases, cancer or mortality amongst those taking β -carotene when compared to a placebo group [15]. These results need to be substantiated further; however, they serve to re-emphasize the fact that there is no substitute for smoking cessation in the prevention of heart diseases.

Table 2 Non-nutrient antioxidants in foods

Product	Anti-oxidant
Soyabean	Isoflavones, phenolic acids
Green tea/black tea	Polyphenols, catechins
Coffee	Phenolic esters
Red wine	Phenolic acid
Citrus and other fruits	Bioflavonoids, chalcones
Onions	Quercetin, kaempferol
Olives	Polyphenols
Mustard/turmeric	Curcumin
Mushrooms	Canthaxanthin

β -carotene and eye disorders

Like vitamins C and E, carotenoids have been associated with decreased cataract risk. Several studies have reported an increased risk of developing cataracts for people with low blood levels of β -carotene. While high intake of fruits and vegetables, rich sources of β -carotene and ascorbic acid, appears to be protective, low intake is associated with increased cataract risk.

Exposure to blue light, in particular, has been implicated in the causation of age-related maculopathy. Since carotenoids absorb blue light, they might be protective against this damage. A significantly reduced risk of macular degeneration has been reported in individuals with high blood carotenoid levels. However, further research is needed to determine conclusively whether carotenoids may reduce the risk of these very prevalent eye disorders in older adults (Table 2).

Other dietary antioxidants

Dietary antioxidants other than vitamin E, vitamin C and the carotenoids may also be

Table 3 Food sources of antioxidant vitamins

Nutrient or non-nutrient	Food Source	Examples
Vitamin E	Vegetable oils	Oils of peanut, soy, palm, corn, safflower, sunflower, cottonseed
	Vegetables	Mustard leaves, turnip greens, pumpkin
	Meat/ fish/ poultry	Chicken liver, salmon, shrimp
	Other	Wheat germ, almonds, peanuts
Vitamin C	Fruits	Citrus fruits, guava, <i>amla</i> (Indian gooseberry), strawberries, melon, pomegranate, papaya
	Vegetables	Tomatoes, leafy vegetables, chillies, cabbage
	Others	Sprouted pulses, germinated cereals
β -carotene	Yellow/ orange vegetables	Carrot, pumpkin, sweet potato
	Yellow/ orange fruits	Apricot, mango, mandarin oranges, oranges, cantaloupe, melons
	Dark green leafy Vegetables	Spinach, mustard leaves, radish, fenugreek leaves, broccoli leaves,

of significance in the prevention of degenerative diseases and the maintenance of good health.

The role of the antioxidant minerals in the etiology of cancer has been investigated and selenium is emerging as a major prophylactic factor against cancer. Low selenium intake has also been linked to myocardial ischaemia and atherosclerosis; however, evidence regarding selenium needs further reinforcement.

There is now convincing evidence that foods containing antioxidants may be of major importance in disease prevention. Garlic has been associated with blood cholesterol lowering properties, cardio-protective aspects, as well as anti-aging properties and this may be attributed to the antioxidants present in it. Apparently, red wine is protective against heart disease and this may be due not to the alcohol content of the wine but to its antioxidant content. *In vitro*, antioxidants isolated from red wine have been found to inhibit oxidation of LDL (Table 3).

Humans require external sources of vitamins E and C and β -carotene as the body is unable to produce these nutrients. Hence, efforts should be made to ensure optimum intake of foods rich in antioxidants. Diets need to be improved and a greater emphasis placed on the consumption of antioxidant rich vegetables and fruits. Authorities in several countries have recommended that at least five servings of fruits and vegetables should be included in the diet daily. Unfortunately, most of our dietary patterns do not lay such an emphasis on vegetables and fruits.

Dietary recommendations for the intake of vitamins E and C and β -carotene currently represent levels suggested to prevent deficiencies in healthy populations. Until recommendations based on the disease prevention abilities of these nutrients emerge, emphasis on promoting consumption of fruits and vegetables may help in reducing the risk of degenerative diseases.

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