Interactions among Antioxidants in Health and Disease: Vitamin E and Its Redox Cycle

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Abstract. **Probably most diseases at some point during their course involve free radical reactions in tissue injury. In some cases, free radical reactions may be involved in multiple sites and at different stages of a chronic disease. So, both acute and degenerative diseases are thought to involve free radical reactions in tissue injury. An overview will be given of the evidence for the occurrence of free radicals and the importance of antioxidant interventions, with particular reference to the lipophilic antioxidant vitamin E (tocopherols and tocotrienols).** [P.S.E.B.M. 1992, Vol 200]

Vitamin E is the name given to a group of naturally occurring lipid soluble antioxidants, the tocopherols and the tocotrienols, that are found naturally in certain plant oils. Since the discovery of vitamin E in Berkeley by H. M. Evans in 1922, when it was first described as an antisterility agent, many scientists and physicians have sought to elucidate its biochemistry, health benefits, and clinical applications.

Vitamin E Paradoxes

Vitamin E is the major, if not the only, chain-breaking antioxidant in membranes, but its membrane concentration is very low, usually equal or less than 0.05–0.1 nmol/mg of protein (less than 1 per 1000–2000 membrane phospholipids). Yet the rate of lipid radical generation in membranes can be very high, about 1–5 nmol/mg protein/min. Nevertheless, under normal conditions, "rancidification," that is oxidation of membrane lipids and proteins, does not occur. Moreover, it is very difficult to render animals deficient in vitamin E, and vitamin E deficiency is seldom found in adult humans. Hence, there must exist a remarkably efficient mechanism for permitting low concentrations of vitamin E to have such high efficiency in protecting membranes against damage and in supporting normal biological activity (1).

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Vitamin E Cycle

We hypothesize that vitamin E acts catalytically, being efficiently reduced from its free radical form, its form after quenching radicals, back to its native state (2).

This catalysis occurs through the interactions between water- and lipid-soluble substances by both nonenzymatic and enzymatic mechanisms, which regenerate vitamin E from its radical (tocotrienoxyl and tocopheroxyl radical back to tocotrienol and tocopherol, respectively) (3). Under conditions where these auxiliary systems act synergistically to keep the steady state concentration of vitamin E radicals low, the loss or consumption of vitamin E is prevented (4–10).

The thioctic acid/dihydrolipoic acid couple is a unique antioxidant system (4). Normally covalently bound lipoamide exists in small amounts as the cofactor of α-ketodehydrogenases in animals. However, larger amounts of fed thioctic acid confer protection in tissues and membranes against oxidative damage. After absorption, thioctic acid may be reduced enzymatically or nonenzymatically to form an active antioxidant. It acts as a double-edged sword in that it appears to interact directly with the membrane to reduce tocopheroxyl radicals (weak effect) or to reduce ascorbate, which in turn acts at membranes to reduce tocopheroxyl radicals (stronger effect). Thus, the thioctic acid/dihydrolipoic acid couple works in recycling vitamin E both in membranes and in low density lipoproteins (LDL), where it acts to stabilize them. The interactions between these antioxidants in membranes and LDL are shown in Figure 1. Thus, vitamin E will only be lost

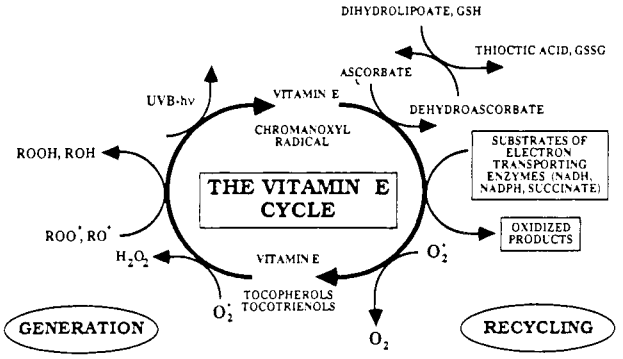

Figure 1. The vitamin E cycle: synergistic action of water- and lipid-soluble antioxidants. Nonenzymatic and enzymatic mechanisms for the regeneration of chromanoxyl radicals.

when these backup systems, either in the aqueous or membrane domains, become rate limiting. At this point, the loss of vitamin E will be accompanied by increased rates of lipid and protein oxidation, destruction of membrane function, and inactivation of membrane enzymes and receptors. Thus, vitamin E not only has an antioxidant action, but also acts as a biological response modifier. By modulating membrane-associated enzyme systems, vitamin E markedly affects the production of small molecular weight substances, like secondary messengers and products of the arachidonic acid cascade, which have profound effects at low concentrations in cell regulation and proliferation (1).

Oxidative damage will be minimized while vitamin E is still present. The concentrations of other antioxidants that act in the cytosol to quench radical reactions or that donate their reducing power to vitamin E may also fluctuate up and down. Membrane-associated redox couples, such as ubiquinol/ubiquinones and oxidized/reduced cytochrome *c*, can also lower the steady state concentrations of vitamin E radicals. Fluctuations in their concentrations, however, are not as important as changes in the concentration of vitamin E. The index of potential damage is high only when vitamin E is gone.

Amplifying the Vitamin E Message: Regulatory Effects of Vitamin E

Intracellular Signaling and Secondary Messenger Formation. Protein kinase C, which is very important in intracellular signaling, may be down-regulated by vitamin E, but not by its esters (11, 12). This would have the effect of inhibiting cell proliferation. These results may be relevant to the anticancer effects of vitamin E.

Production of Cell Mediators by the Arachidonic Acid Cascade. Phospholipase A_2 preferentially hydrolyzes peroxidized fatty acid esters of phospholipid membranes. Since lipid peroxides activate phospholipase A_2, a decrease in lipid peroxides by vitamin E will decrease phospholipase A_2 activity (13, 14). Vitamin E thereby modulates arachidonic acid release from membrane phospholipids and, therefore, arachidonic acid metabolism.

Vitamin E also inhibits plant and mammalian 5- and 15-lipoxygenases (15–17). Fatty acid hydroperoxides necessary for activation of lipoxygenase can overcome the inhibition. Finally, prostaglandin and hydroxyeicosatetraenoic acid production from arachidonic acid in bovine seminal vesicles and kidney was inhibited by α-tocopherol; hydroxyeicosatetraenoic acid production was inhibited less than that of prostaglandin. Thus, vitamin E seems to influence both the cyclo-oxygenase and lipoxygenase pathways; this modulation of arachidonic acid oxidation may have important *in vivo* implications.

Hypocholesteremic Effects. Tocotrienols, the form of vitamin E with an unsaturated isoprenoid side chain, can serve as powerful hypocholesterolemic agents. The γ-form is most active. These effects are not exhibited by the corresponding tocopherols. Tocotrienol may well act by inhibiting human menopausal gonadotropin CoA reductase activity (18), the rate-limiting step in the biosynthesis of cholesterol.

Anticancer Effects. Mevalonate-derived intermediates of sterol biosynthesis, including tocotrienols from red palm oil, suppress neoplastic growth (19). Increased linoleic acid content in fat diets also suppresses tumor growth. The growth of several types of transplantable tumors is also inhibited by tocotrienols, and particularly by the γ-isomer (20).

Thus, modulation of tumor growth rate by substances with polyunsaturated chains has potential anticancer activity. The extent to which these effects are mediated through actions on prostanoid formation or on human menopausal gonadotropin CoA reductase activity is still unclear.

Role of Vitamin E in Degenerative and Coronary Artery Disease

Several epidemiological investigations in recent years have provided strong circumstantial evidence for beneficial effects of vitamin E in degenerative and coronary heart disease (Table I).

Ischemic Heart Disease Mortality. The World Health Organization/Monica core study (27), carried out with 20 populations in Europe, is the most recent, extensive, and best controlled epidemiological investigation thus far conducted on risk factors involved in death from ischemic heart disease (IHD). In this study, a combination of classical risk factors accounted for only 20% of IHD mortality. A good correlation was found between elevated plasma cholesterol and IHD mortality in 12 populations, but not in eight others. Of various measured parameters, the best correlation was found between low plasma concentrations of vitamins E and A and increased IHD mortality (27).

Table I. Health Benefits and Clinical Applications of Vitamin E

Clearly defined need	
Malabsorption	Cholestatic liver disease, cystic fibrosis, abetalipoproteinemia, celiac disease, sprue, pancreatitis
Familial deficiency	Genetic factors
Prolonged total parenteral nutrition	Environmental factors
Some evidence, not completely accepted	
Premature infants	Reduced risk of intraventricular hemorrhage and reduced severity of retrolental fibroplasia
Intermittent claudication	Increased mobility and stronger exercise endurance
Peyronie's disease	Decreased fibrosis, pain
Hemolytic anemias	Sickle cell, glucose-6-phosphate dehydrogenase deficiency, thalassemia
Other indications	
Adult respiratory distress syndrome (ARDS)	Low plasma E levels in ARDS suggest a need for parenteral E administration, which may delay onset of acute respiratory failure (21)
Epilepsy	Vitamin E supplementation in addition to antiepileptic drug therapy resulted in reduction in seizures in a majority of epileptic children refractory to antiepileptic drugs (22)
Tardive dyskinesia	After vitamin E administration, scores on Abnormal Involuntary Movement Scale were lower in subjects with persistent tardive dyskinesia (23)
Cancer therapy	Radiotherapy. Plasma E and β-carotene levels were decreased in patients during radiotherapy prior to bone marrow transplantation. Antioxidant loss must be considered a possible cause of early posttransplant organ toxicity (24)
	Chemotherapy. Heart damage due to adriamycin therapy was decreased by vitamins A and E (25)
Burns	Vitamin E supplementation stimulated T helper cells to near normal levels in patients after burn injury (20–64% of body surface area) (26)

IHD mortality was highest in the extreme northern countries, e.g., Scandinavia, moderate in middle European countries, and lowest in the countries of southern Europe bordering the Mediterranean region. A 7-fold higher IHD was found in populations in the north as compared with the south. IHD mortality was inversely related to the amount of yellow and green vegetables in the diet of these European populations. When the data from the 12 cholesterol-sensitive populations cited above were adjusted for cholesterol- and lipid-standardized factors, a lower risk to IHD mortality was still significantly correlated with higher plasma levels of vitamins E and A.

Angina Pectoris. In a recent epidemiological study (28), the hypothesis was tested that plasma concentrations of antioxidant vitamins might be related to the risk of angina. Classic risk factors for coronary heart disease that were evaluated included age, habitual smoking, blood pressure, lipid composition, relative body weight, and also seasonal trends in vitamin and antioxidant concentrations in plasma samples from 6000 men aged 35–54. Without adjustment for the various risk factors, plasma concentrations of vitamin E, vitamin C, and carotenoids were inversely related to an increased risk of angina pectoris. However, after adjustment for the various risk parameters, only plasma vitamin E remained as a significant factor. The adjusted odds ratio for angina between the lowest and highest quantities for vitamin E was 2.68 ($P = 0.02$). Thus, populations with a high incidence of coronary heart disease might well benefit from diets rich in vitamin E.

Coronary Artery Disease: Atherosclerosis. Cholesterol-rich low density lipoproteins are clearly involved in the multistep process of coronary artery disease. Many years ago, atherosclerotic lesions were shown to occur in rabbits fed diets high in cholesterol. Feeding with antioxidants, such as vitamin E, reduced the occurrence of these lesions. Polyunsaturated fatty acids, which are susceptible to lipid peroxidation, can form free radicals that can injure the endothelium, damage heart muscle cells, and provoke proliferation of smooth muscle. These processes are inhibited by vitamins C and E.

When polyunsaturated fatty acids, cholesterol, and/or apoprotein B in low density lipoproteins are oxidatively modified, the damaged LDL are phagocytized by macrophages. Extensive uptake of damaged LDL by macrophages results in the formation of foam cells. Foam cells accumulate under the unbroken layer of endothelial cells, where they aggregate to form a fatty streak. The fatty streak serves as a nucleus for the development of the atherosclerotic plaque. Higher vitamin E concentrations would be expected to retard all of these processes. Thus, the multifactorial actions of vitamin E, not only as an antioxidant but also as a biological response modifier, account for the high correlation between low plasma levels of vitamin E and the high risk of coronary artery disease.

Benefits of Vitamin E in Other Chronic Diseases and in Aging

Aging. Deposits resulting from free radical reactions accumulate in tissue during the aging process. Lipid peroxidation may be an important factor in provoking premature aging.

Cancer. Reactive oxygen species have been implicated in the process of cancer initiation and promotion. Vitamin E and the other antioxidants can function as anticarcinogens by quenching free radicals or reacting with their products. Epidemiological studies indicate that vitamin E, alone or in combination with other antioxidants, is associated with a decreased incidence of certain forms of cancer.

Skin cancer is both the most common form of human cancer and the most common malignant cancer. Populations exposed to high levels of ultraviolet irradiation show an increased incidence of malignant and nonmalignant melanoma. Alarmingly, the incidence of this disease has doubled in the United States in the past decade. Because antioxidants retard the multistep process of carcinogenesis, molecular damage due to ultraviolet irradiation may well be reduced by vitamin E.

Anticancer agents that generate reactive oxygen species, such as adriamycin, damage normal tissue. Thus, in patients treated with adriamycin (doxorubicin), vitamin E may be beneficial in protecting the skin, the heart, and other tissues.

Arthritis. Increased free radical production has been observed in animal and human studies on arthritis. Vitamin E therapy is effective in relieving pain and in improving mobility in patients with osteoarthritis.

Circulatory Conditions. Excessive platelet aggregation speeds the development of atherosclerosis. Vitamin E supplementation significantly decreases platelet aggregation in healthy adults and reduces elevated platelet aggregation rates in patients with high blood lipid levels and in oral contraceptive users. In patients with coronary artery disease who undergo bypass surgery, pretreatment with vitamin E reduces the elevated free radical concentrations in the plasma that usually occur.

Cataracts. Photo-oxidative mechanisms and reactive oxygen species are important in cataractogenesis. Vitamin E delays or minimizes cataract development in isolated animal lenses. High plasma antioxidant concentrations correlate with reduced cataract risk in adults.

Exercise. Strenuous physical exercise is associated with an increased rate of lipid and protein oxidation and of vitamin E consumption. Mountain climbers who were given oral doses of 400 IU/day of vitamin E showed improved physical performance and decreased breath pentane output during prolonged exposure to high altitudes.

Air Pollution. Vitamin E is an important component of the lung's defense against the injurious effects of smog, smoke, and smoking.

Relative Activities of Tocopherols and Tocotrienols

D-α-Tocopherol and D-α-tocotrienol, two forms of vitamin E, are natural membrane antioxidants. Both have the same aromatic chromanol "head," but D-α-tocopherol has a saturated whereas D-α-tocotrienol has an unsaturated polyisoprenoid "tail."

In membranes, D-α-tocotrienol shows 40 to 60 times higher antioxidant potency than D-α-tocopherol due to: (i) higher recycling efficiency from chromanoxyl radicals; (ii) more uniform membrane bilayer distribution; and (iii) better interaction of chromanols with lipid radicals (29). Thus, D-α-tocotrienol may have higher physiological activity than D-α-tocopherol under conditions of oxidative stress.

Both D-α-tocopherol and D-α-tocotrienol protect human LDL against oxidative modification induced by lipoxygenase, UV irradiation, or peroxyl radicals initiated by azo dyes. As revealed by electron spin resonance spectroscopy, chromanoxyl radicals of both D-α-tocopherol and D-α-tocotrienol are formed in oxidatively stressed LDL. In the presence of ascorbate, the recycling efficiency for D-α-tocotrienol was higher than for D-α-tocopherol.

In myocardial ischemia-reperfusion studies (30), palm oil vitamin E (POE) (a gift of A. Gapor, PORIM), which contains 45% D-α-tocopherol and 55% D-α-tocotrienol, conferred protection to rat hearts against ischemia-reperfusion injury. After 40 min of ischemia, the hearts from animals fed POE for 45 days recovered 90% of the control value of their mechanical activity (contractility), whereas hearts from the control group recovered only 17% of the initial level.

Lipid and protein oxidation in the reperfused heart was greatly reduced by POE treatment. During the reperfusion period, more D-α-tocotrienol (79%) than D-α-tocopherol (59%) was consumed. D-α-Tocotrienol also demonstrated higher *in vitro* NADH-, NADPH-, succinate-, and ascorbate-dependent recycling efficiency from chromanoxyl radicals in heart membranes (mitochondria and microsomes) than D-α-tocopherol.

Thus, D-α-tocotrienol clearly is highly efficacious in protecting myocardial membranes against oxidation in the course of ischemia-reperfusion injury.

New Horizons in Vitamin E Research: Requirements for Vitamin E

Vitamin E Paradox. How do we reconcile the great efficiency of minute quantities of vitamin E in membranes with the beneficial effects of vitamin E supplementation on human health seen in chronic diseases and acute clinical conditions?

A local deficiency of vitamin E can arise rapidly in

membranes under conditions of intense oxidative stress. Under these conditions, molecular damage to lipids, proteins, and nucleic acids can be expected. Examples of such acute situations are ischemia-reperfusion injury, e.g., myocardial infarction or stroke and subsequent reoxygenation, hemorrhagic and other forms of shock, exposure to extremes of environmental pollution or irradiation, and treatment with antineoplastic drugs.

The replenishment of vitamin E in depleted membranes often requires days to weeks, even in tissues in which vitamin E turnover is rapid. Thus, dietary supplementation with vitamin E during or after an episode of acute stress may prevent tissue injury.

Because water-soluble antioxidants that recycle vitamin E can be introduced in a matter of minutes, the effective concentration of the residual vitamin E in membranes or lipoproteins thereby may be rapidly increased. Similarly, water-soluble recyclers or water-soluble forms of vitamin E, such as vitamin E phosphate or vitamin E succinate, may also be more effective than vitamin E itself. Thus, the concept of vitamin E recycling may prove to be crucial in the development of new strategies for the treatment of acute conditions involving oxidative damage.

Biokinetics and Tissue Absorption of Vitamin E. The biokinetics and tissue absorption of different forms of vitamin E in humans have not been well studied. On the basis of animal studies, however, fast and slow turnover tissues have been identified. Furthermore, certain tissues, such as adipose tissue, the liver, and the adrenal gland, accumulate vitamin E.

Dietary vitamin E is transported on chylomicra from the intestine to the liver and other tissues. In rat liver, an α-tocopherol-binding protein has been identified; however, its specificity for the different tocopherols, i.e., α, β, γ, and δ, and for the corresponding tocotrienols is unknown. Whether this binding protein is involved in the incorporation of vitamin E into lipoproteins is also unclear. In human plasma, tocopherols tend to be associated with phospholipid-rich lipoproteins, whereas tocotrienols are primarily found in triglyceride-rich lipoproteins (K. C. Hayes, personal communication). Thus, different pathways must exist for the incorporation of these two forms of vitamin E into lipoproteins. In regard to tissue uptake, adipose tissue becomes enriched in tocotrienols, while most other tissues contain more α-tocopherol than α-tocotrienol. Thus, specificity is also expressed at the interface between plasma lipoproteins, probably including chylomicra, and various tissues.

In our laboratory, rats fed large supplements of POE with roughly equivalent concentrations of tocopherol and tocotrienol accumulated both tocopherols and tocotrienols in many tissues, different skeletal muscle fibers, and liver. Future biokinetic and tissue absorption studies in both humans and animals should allow us to define better the biological functions and actions of tocopherol and tocotrienol isomers.

Vitamin E Requirement. Health status, lifestyle, diet, and environment markedly influence the requirements for vitamin E. Although demonstrable vitamin E inadequacy in apparently healthy adults is rare, vitamin E requirements may vary 5-fold in individuals, depending on the dietary intake of polyunsaturated fat, tissue composition, the steady state concentrations of other interactive antioxidants in tissues, and genetic factors.

Free radical-mediated damage has been implicated in cellular and extracellular changes that occur over time in the aging process and in the development of chronic diseases. Vitamin E and other antioxidants prevent or minimize oxidative damage in biological systems. How adequate the antioxidant defense should be to protect the body from excessive free radical concentrations is one of the many new horizons for vitamin E research.

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