Recent advances of vitamin E pathophysiology

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Vitamin E was discovered over 75 years ago, yet it has been only recently recognized that human vitamin E deficiency occurs as a result of fat malabsorption syndromes, defects in lipoprotein metabolism, and defects in the gene for the α-tocopherol transfer protein. Although the frequency of human vitamin E deficiency is unknown, it is likely that it is very rare. In individuals at risk, it is clear that vitamin E supplements should be recommended to prevent deficiency symptoms. What about their use in normal individuals? Vitamin E supplementation in normal individuals is quite controversial. It has been assumed that usual dietary vitamin E intakes are adequate because human vitamin E deficiency is rare and experimental vitamin E deficiency difficult to produce in laboratory animals. A continuing problem in nutrition is whether nutrients have beneficial effects when consumed in amounts in excess of those 'required' by the body. For most vitamins, excess amounts are wasted and provide no added benefits. Indeed, some fat soluble vitamins can be stored and excess amounts become toxic. Antioxidant nutrients may, however, be different. Heart disease and stroke, cancer, chronic inflammation, impaired immune function, Alzheimer's disease: a case can be made for the role of oxygen-free radicals in the etiology of all of these disorders and even in aging itself. Do antioxidant nutrients counteract the effects of free radicals and thereby ameliorate these disorders? And if so, do large antioxidant supplements have beneficial effects beyond 'required' amounts or even in amounts beyond those that could be obtained from a well-balanced diet? These are questions for which not only scientists, but also the public, are eagerly awaiting the answers.

Key words: tocopherol, tocotrienol, heart disease, stroke, cancer, chronic inflammation, impaired immune function, Alzheimer's disease.

Vitamin E biologic activity

Vitamin E occurs in nature as α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols. The biologic activity of each of the forms is dependent upon its absorption, lipoprotein transport, delivery to tissues, and metabolism.[1] Apparently all of the various vitamin E forms are similarly absorbed, secreted in chylomicrons into the circulation and during chylomicron catabolism transferred to tissues. Vitamin E structure is important in the liver. The hepatic α-tocopherol transfer protein (α-TTP) selectively chooses α-tocopherol for enrichment of nascent very low density lipoproteins (VLDL). During VLDL catabolism in the circulation, α-tocopherol is transferred to all of the plasma lipoproteins. In this manner, the liver regulates plasma vitamin E concentrations and is responsible for replacing nearly the entire plasma pool of vitamin E daily. Vitamin E is delivered to tissues by three mechanisms: delivery of vitamin E during lipolysis; as a result of lipoprotein uptake; and as a result of exchange of vitamin E between lipoproteins and tissues. Regulation of tissue vitamin E is not well understood. Much remains to be learned about vitamin E functions and about vitamin E tissue regulatory mechanisms in response to oxidative stress.

Vitamin E deficiency in humans

Vitamin E deficiency is quite rare in humans, but does occur as a result of genetic abnormalities in α-TTP and as a result of various fat malabsorption syndromes. Human vitamin E deficiency symptoms are characterized by a progressive peripheral neuropathy with a specific 'dying back' of the large caliber axons of the sensory neurones, which results in incoordination and an inability to walk.[2] Here it is important to recognize that patients with peripheral neuropathy should be tested for vitamin E deficiency.[3]

Genetic abnormalities in hepatic alpha-tocopherol transfer protein

Defects in the α-TTP gene are associated with a characteristic syndrome, ataxia with vitamin E deficiency (AVED, previously called familial isolated vitamin E (FIVE) deficiency).[4] Ataxia with vitamin E deficiency patients have neurologic abnormalities which are similar to those of Friedreich's ataxia.[3,5,6] There have been approximately 100 people in the world who have been found to have α-TTP gene defects; most of the patients have been found in Tunisia and described by Ben Hamida *et al.*[5,6] In addition, Gotoda *et al*. characterized the population of a remote Japanese island, home of an AVED patient homozygous for an α-TTP gene defect, and found 22 heterozygotes.[7]

AVED patients are responsive to oral vitamin E supplements. A dose of 800–1200 mg/day is usually sufficient to prevent further deterioration of neurologic function and in

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some cases improvements have been noted, as reviewed by Sokol.[2] Untreated patients have extraordinarily low plasma vitamin E concentrations (as low as 1/100 of normal), but if they are given vitamin E supplements, then plasma concentrations reach normal within hours. However, if supplementation is stopped, then plasma vitamin E concentrations fall within days to deficient levels.

Traber *et al.* described the biochemical defect in AVED patients using various vitamin E forms labelled with stable isotopes.[8,9] Controls maintained plasma d6-α-RRR-tocopherol concentrations by preferentially secreting it in VLDL, while patients had an impaired secretion of α-tocopherol in VLDL. Furthermore, some patients could not discriminate between various forms of vitamin E. Thus, it appeared that firstly, an hepatic α-tocopherol transfer protein, which preferentially incorporates RRR-α-tocopherol into VLDL, is required to maintain plasma RRR-α-tocopherol concentrations via secretion in nascent VLDL by hepatocytes; secondly, that some patients are lacking this protein, or have a marked defect in the RRR-α-tocopherol binding region of the protein; and thirdly, that some patients have a less severe defect, perhaps a defect in transfer function.

Genetic abnormalities in lipoprotein metabolism

Vitamin E deficiency is also caused by genetic defects in lipoprotein metabolism. Studies of patients with hypobetalipoproteinemia or abetalipoproteinemia (low to non-detectable circulating chylomicrons, VLDL or low density lipoproteins (LDL) have demonstrated that lipoproteins containing apoB are necessary for effective absorption and plasma transport of lipids, especially vitamin E.[10] These patients have steatorrhea from birth because of their impaired ability to absorb dietary fat, which also contributes to their poor vitamin E status. Again, this is a relatively rare disorder; about 100 people in the world have been found to have extremely low to absent apoB-containing lipoproteins, but unlike AVED no large concentration of such patients have been found.

Homozygous hypobetalipoproteinemia patients have a defect in the apoB gene and thus any apoB-containing lipoproteins that are secreted into the circulation are cleared rapidly due to the defective apoB.[11] Abetalipoproteinemic patients have genetic defects in MTP (microsomal triglyceride transfer protein), which prevents normal apoB synthesis and thus the secretion of apoB-containing lipoproteins is virtually nonexistent.[12] Clinically, both groups of subjects become vitamin E deficient and develop a characteristic neurologic syndrome, a progressive peripheral neuropathy, if they are not given large vitamin E supplements.[10] Doses of 100–200 mg/kg, or about 5–7 g of vitamin E per day, are recommended.[2]

Fat malabsorption syndromes

Vitamin E deficiency also results from fat malabsorption syndromes. Vitamin E deficiency occurs secondary to fat malabsorption because vitamin E absorption requires biliary and pancreatic secretions. Failure of micellar solubilization and malabsorption of dietary lipids leads to vitamin E deficiency in children with chronic cholestatic hepatobiliary disorders, including disease of the liver, and intrahepatic and extrahepatic bile ducts.[2] Children with cholestatic liver disease, who have impaired secretion of bile into the small intestine, have severe fat malabsorption and demonstrate neurologic abnormalities as early as the second year of life. These neurologic abnormalities become irreversible if the vitamin E deficiency is left uncorrected.[2]

Children with cystic fibrosis can also become vitamin E deficient because the impaired secretion of pancreatic digestive enzymes causes steatorrhea and vitamin E malabsorption, even when pancreatic enzyme supplements are administered orally.[2] More severe vitamin E deficiency occurs if there is, in addition, an impairment in bile secretion. Winklhofer-Roob *et al.* proposed that cystic fibrosis patients should be supplemented with 400 mg vitamin E so that their plasma α-tocopherol concentrations increase above 26–28 μmol.[13,14] This higher plasma vitamin E was found to protect LDL from *in vitro* oxidation.

It should be emphasized that any disorder that causes fat malabsorption can lead to vitamin E deficiency. The list of disorders associated with acquired vitamin E deficiency assembled by Sokol includes chronic dysfunction or resection of the small bowel, inflammatory bowel disease, Crohn's disease, mesenteric vascular thrombosis or intestinal pseudo-obstruction, blind loop syndrome, intestinal lymphangiectasia, celiac disease and chronic pancreatitis.[2] The development of neurologic symptoms of vitamin E deficiency in adults with these disorders takes decades. Serum vitamin E may fall within 1–2 years of acquired lipid malabsorption in adolescents and adults; however, a 10–20 years interval between the identification of biochemical vitamin E deficiency and the onset of neurological symptomatology is generally observed in adults. The prolonged time for onset of symptoms results from the prior accumulation of vitamin E in most tissues and its relatively slow depletion from nervous tissues.

Premature infants

Newborn infants, especially premature babies, have a low vitamin E status[15,16] and premature babies respond very slowly to dietary vitamin E intakes.[17]

It has been suggested that vitamin E deficiency contributes to alterations in neonatal neutrophil function through peroxidative damage to cell membranes.[18] These defects may play a role in determining the high susceptibility of the newborn infants to infections as a result of host defense impairment. Chirico *et al.* administered a total of 120 mg/kg *all rac*-α-tocopherol in divided doses to 10 of 20 healthy premature infants and assessed neutrophil phagocytosis.[19] In the treated and untreated infants no differences were found in neutrophil function before treatment with vitamin E although phagocytosis, bactericidal activity, and chemotaxis were lower than in 30 adult controls. During their first week of life, the untreated infants maintained a low index and frequency of phagocytosis while these parameters were significantly increased in the group receiving vitamin E. However, after 2 weeks of age, phagocytosis was normal in both groups of infants with no differences being noted in bactericidal activity, chemotaxis or metabolic activity. Thus, vitamin E accelerated the normalization of phagocytic function during the first week of life in these premature infants.

Retinopathy of prematurity has been thought to have an oxidative stress component and vitamin E has been used as

an ameliorative. However, the lack effectiveness in preventing severe cases of retinopathy of prematurity and the toxicity of vitamin E supplementation in high-risk premature infants is still disputed.[20, 21]

Vitamin E supplementation in oxidative stress

Oxidative stress is increasingly recognized as an adverse factor in ageing, and in a large number of chronic diseases such as heart disease,[22,23] cancer,[24] diabetes[25] and Alzheimer's disease.[26] Oxidative stress is a shift in the balance between cellular oxidants and antioxidants towards the former.[27] The major source of these reactive oxygen species and radicals in the body is the 'leakage of electrons' during oxidative metabolism for conversion of foodstuffs to energy.[28,29]

Organisms have evolved a variety of mechanisms for guarding against an excess of oxygen free radicals. Indeed, reactive oxygen species 'turn on' various signal transduction pathways eliciting cellular responses, especially defense mechanisms.[30–32] The interplay of antioxidants, antioxidant enzyme systems and the ability to generate reducing equivalents serve to protect and defend the organism.[33] The dependence of this defensive mechanism on low molecular weight antioxidants raises the question: 'Are supplemental antioxidants beneficial in protecting against oxidative stress?'

Cardiovascular disease

Perhaps the most convincing evidence for the role of oxidative stress and protection by antioxidants in the disease process is that for heart disease. Here, it has been proposed that atherosclerosis results from damage to the lining of the artery walls caused by the oxidation of LDL and their sequelae.[22]

Oxidatively modified LDL are most likely the main source of the cholesterol that accumulates in atherosclerotic plaques.[34–36] The evidence for the participation of LDL oxidation in atherosclerosis includes: oxidized LDL are present in atherosclerotic lesions;[37] *in vitro* experiments show increased uptake of oxidized LDL by macrophages;[38] oxidized LDL are metabolized by macrophages and smooth muscle cells via the scavenger pathway that, unlike the native LDL receptor, are not down regulated;[38,39] and oxidized LDL cause injury to cultured cells.[40] Oxidized LDL also alter growth factor and cytokine production,[41,42] induce monocyte recruitment and adhesion to endothelium,[43,44] and alter cell migration and growth.[38,45,46]

It is therefore likely that LDL enter the endothelial lining where they become oxidized, leading to invasion of monocytes and their transformation into foam cells and ultimately atheroma. If this process is dependent upon LDL oxidation, then prevention of LDL oxidation should prevent, or at least limit atheroma formation. Indeed, in the test tube LDL isolated from subjects consuming supplemental vitamin E are less oxidizable.[47–50] In a placebo-controlled, double-blind study there was a 77% reduction of non-fatal heart attacks by supplementation with 400–800 IU vitamin E, providing further evidence in support of the concept of antioxidant supplementation in the protection against oxidative stress in atherosclerosis.[51] This concept is also supported by epidemiologic studies showing that vitamin E supplementation is associated with a decreased risk of chronic heart disease.[52,53] Lower stenosis scores are also correlated with higher LDL vitamin E.[54]

Tocotrienols have been demonstrated to protect LDL from oxidative modification.[55] An *in vivo* test of the use of tocotrienols in patients with hyperlipidemia and carotid stenosis by Tomeo *et al.*[56] demonstrated a significant regression in the carotid atherosclerotic plaque in nine out of 25 patients treated with Palmvitee (16 mg α-tocopherol, 40 mg γ- and α-tocotrienols), two with progression and no progression in the remaining patients. In contrast, none of the control group exhibited regression, and 10 of 25 had further progression. These data suggest that vitamin E in Palmvitee decreased the *in vivo* oxidative stress and thereby enhanced atheroma regression.

Inhibition of cholesterol synthesis by γ-tocotrienol. Tocotrienols increase the rate of degradation of 3-hydroxy-3-methyl glutaryl coenzyme A (HMGCoA) reductase protein, the rate controlling step in cholesterol biosynthesis.[57–59] Furthermore, in the presence of lovastatin, a drug that competitively inhibits HMGCoA reductase, the amount of protein and its activity were both suppressed by the presence of γ-tocotrienol in the incubation medium of HepG2 cells.[58] These data suggest that γ-tocotrienol enhances the degradation of the protein that controls cholesterol synthesis. This study further suggests that a hypocholesterolemic effect of γ-tocotrienols should result from a decrease in cholesterol synthesis and an increase in LDL receptor activity. Khor *et al.* demonstrated that tocotrienols accumulate in the liver, not the plasma, of guinea pigs administered tocotrienol-rich-fraction of palm oil (TRF) by intraperitoneal injection.[60] Since most of the cholesterol synthesis takes place in the liver, tocotrienol accumulation here should effectively limit cholesterol synthesis *in vivo*.[61]

Qureshi *et al.* first reported that tocotrienols inhibited cholesterol synthesis in chickens.[62] Subsequently, they demonstrated that Palmvitee, as well as γ-tocotrienol, had potent hypocholesterolemic effects in hypercholesterolemic humans.[63] Similarly, Tan *et al.* showed that Palmvitee lowered both serum total cholesterol (5.0% to 35.9%) and LDL-cholesterol (0.9% to 37.0%) concentrations in all the volunteers.[64] However, there are conflicting reports in humans concerning the hypocholesterolemic effects of tocotrienol supplements.[65–67] It has been suggested that the lack of effect of tocotrienols on plasma cholesterol levels results from use of mixtures of tocotrienols and tocopherols.[66, 68]

Low fat diets. Individuals with elevated cholesterol levels often change their dietary habits, increasing intakes of polyunsaturated fat (PUFA) and decreasing saturated fat. However, these changes may have deleterious effects on vitamin E intakes because most dietary vitamin E is present in fats.[69] To avoid excessive intakes of PUFA, the ingestion of mono-unsaturated fats, such as olive or canola oils, is currently recommended.[70] Palm oil may be also be an acceptable alternative to high PUFA fats because it contains 40% mono-unsaturated fat[71] and as reviewed by Sundram, palm oil does not raise plasma cholesterol.[72] A direct comparison of the effects of palm oil and olive oil on plasma lipids demonstrated that neither raised plasma cholesterol.[73] Zhang *et al.* reported that a 30% fat diet containing approximately 80% of the fat from palm oil resulted in serum lipid concentrations similar to those of subjects eating either soybean oil or

peanut oil as their major fat sources.[71] Palm oil has the major benefit that it contains tocotrienols and α-tocopherol as its major vitamin E forms. Nazaimoon *et al.* showed that in diabetic subjects consuming a palm oil-containing diet, the amount of vitamin E present in palm oil was sufficient to significantly decrease lipid peroxidation *in vivo*.[67]

Taken together, these data suggest that vitamin E has multiple potential mechanisms by which it could ameliorate or reverse atheroma formation. Specifically, it has potent antioxidant activity so may inhibit LDL oxidation and its sequelae. Further, some vitamin E forms may inhibit cholesterol synthesis, and increase LDL receptor activity, thereby lowering serum cholesterol. These potential benefits suggests that further investigation of the health benefits of tocotrienols are warranted.

Cancer

Free radical damage is thought to be involved in the initiation and promotion of many cancers. The increased incidence of cancer among older adults has been postulated to be due, in part, to the increasing level of free radical reactions with age and the diminishing ability of the immune system to eliminate the altered cells. The efficacy of vitamin E in the prevention and treatment of different forms of cancer and the role of the immune system in such actions remain equivocal.

Epidemiological data suggest that high intakes of vitamin E (and other dietary antioxidants) may decrease the risk for certain cancers, particularly cancers of the breast, colon, lung, and stomach.[74] Knekt *et al.* assessed blood vitamin E levels and subsequent cancer incidence in a longitudinal study of 21 172 men in Finland.[75] Vitamin E was measured from stored blood samples of 453 subjects who developed cancer during the 6–10 years study period and 841 matched controls. Adjusted relative risks in the two highest quintiles of blood vitamin E concentrations compared to all other quintiles were 0.7 for all cancers and 0.6 for cancers unrelated to smoking.

Randomized, double-blind, placebo-controlled clinical trials of vitamin E supplements offer the most definitive opportunity to clarify vitamin E's potential in cancer prevention. Only three large trials of this kind have published their results. In the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Trial, 29 133 male current smokers in Finland, aged 50–69 years and with a median duration of cigarette use of 36 years, were randomized to 50 mg of synthetic dL-α-tocopherol daily, β-carotene, α-tocopherol and β-carotene, or placebo.[76,77] After 5–8 years of follow-up, lung cancer incidence was not reduced by supplemental vitamin E (433 vs. 443 cases), nor was total cancer mortality (7.8% increase; 579 vs. 537 deaths). However, prostate cancer incidence was statistically significantly reduced by 34% (99 vs. 151 cases), colorectal cancer incidence was modestly reduced by 16% (68 vs. 81 cases), while bladder and stomach cancer incidence were increased by 9% and 25% (81 vs. 74 and 70 vs. 56 cases, respectively). Compliance was excellent; after three years, serum α-tocopherol concentrations (median at baseline = 11.5 mg/mL) were consistently increased by 50%. Further analysis of the ATBC study demonstrated that long-term supplementation with α-tocopherol reduced prostate cancer incidence (32%) and mortality (41%) in male smokers.[78]

Inhibition of cancer cell proliferation by tocotrienols. Cholesterol synthesis precedes DNA synthesis in dividing cells.[79–81] However, Elson and Yu suggest the HMGCoA reductase in neoplastic tissues is markedly resistant to sterol feedback inhibition, but the mevalonate pathway of tumour tissues is uniquely sensitive to the inhibitory actions of the dietary isoprenoids.[82] According to Elson and Qureshi, the impact of palm oil on cardiovascular disease and cancer may be explained by the mevalonate-suppressive action of constituent isoprenoid end products of plant secondary metabolism.[83] Guthrie *et al.* report that tocotrienols in combination with tamoxifen and with various flavonoids inhibit breast cancer cell proliferation.[84,85] This synergism suggests that multiple mechanisms are involved and that tocotrienols and flavonoids may be a potent combination in the inhibition of cancer cell proliferation.

Inhibition of cell proliferation by alpha-tocopherol. Protein kinase C activity is inhibited by α-tocopherol but not by β-tocopherol, suggesting a central role of this enzyme in the control of cell proliferation by α-tocopherol.[86–88] Activation of the transcription activation complex AP-1 (but not NF-kB) is prevented by α-tocopherol and not by β-tocopherol. However, Chatelain *et al.* found that the tocotrienols were toxic in this system, suggesting that tocopherols and tocotrienols work by different mechanisms.[89]

Diabetes

Protein glycation, a nonenzymatic reaction involving sugar, appears to play a role in the evolution of diabetic complications such as retinopathy, neuropathy, renal failure and atherosclerosis.[90] Jain and Palmer suggest that increased oxygen radicals can initiate peroxidation of lipids and malondialdehyde (MDA) accumulation, which in turn, can stimulate glycation of proteins in diabetes.[91] They found that *in vitro* vitamin E can block the glycation of proteins by inhibiting MDA formation. Perhaps more importantly, Jain *et al.* demonstrated that 35 diabetic patients, who were supplemented with either all *rac*-α-tocopherol (100 IU/day) for 3 months or a placebo in a double-blind clinical trial, had a significant reduction in glycated haemoglobin[92] and lipid peroxidation.[93]

Abnormal polymorphonuclear cell function including defective phagocytic uptake and chemotactic responses, and excess superoxide anion production, has been reported in diabetics.[94] Hill *et al.* administered 25 IU α-tocopherol/kg/day orally for several weeks to seven diabetic patients with consistently depressed monocyte chemotactic responses.[95] The vitamin E treatment doubled monocyte random motility and chemotactic responsiveness to levels comparable to normal controls, suggesting that this defective function may partially be a result of auto-oxidative membrane damage. Interestingly, vitamin E supplementation at 900 mg/day for 4 months also improved insulin action in healthy subjects and diabetic patients.[96]

Parkinson's Disease and Alzheimer's Disease

Afflictions of the nervous system, in general, are of interest with respect to vitamin E therapy in view of the neurochemical, neurophysiological and neuropathological information obtained from studies of vitamin E deficiency.[2,97] For example, there is evidence linking oxygen free radicals and Parkinson's disease.[98] However, the serum levels of vitamin

E and the vitamin E/cholesterol ratios were found not to be significantly lower in the patients with Parkinson's disease than in controls.[99] Furthermore, the conclusion from the DATATOP study (a multicentre trial of more than 800 patients) was that Deprenyl (10 mg per day) but not vitamin E (2000 IU per day) delays the onset of disability associated with early, otherwise untreated Parkinson's disease.[100] In an analysis of the brain levels of Alzheimer's disease patients and of Parkinson's disease patients,[101] it was found that the tocopherol levels in the midbrain were doubled in both these groups as compared to controls, and that there were no differences between the two groups of patients. In contrast, Dexter *et al.* studied four patients with vitamin E deficiency and sensory ataxia and found reduced [^{18}F]dopa uptake in both putamen and caudate in the two most severely affected patients.[102] They concluded that severe and prolonged vitamin E deficiency results in loss of nigrostriatal nerve terminals and supports the hypothesis that oxidative stress may contribute to the aetiology of Parkinson's disease.

Regarding Alzheimer's disease, experimental work has shown that metal-catalysed oxidation confer amyloidogenicity to amyloid protein precursor fragments,[103] and vitamin E protects nerve cells from amyloid β protein toxicity.[104] Furthermore, in patients with moderately severe impairment from Alzheimer's disease, treatment with selegiline or α-tocopherol slowed the progression of the disease.[105]

The role of vitamin E in neurologic disorders is an important one and requires further study.

Environmental factors and skin

Skin, the outermost barrier of the body, is exposed to oxidative stress from a variety of environmental insults, including ultraviolet (UV)-irradiation, ozone, halogenated hydrocarbons, and smoke. A variety of enzymatic and nonenzymatic antioxidants protect skin against environmental pollutants and oxidative stress.[106–110] Among these, vitamin E appears to play a pivotal role.

UV-light. During oxidative stress caused by prolonged UV-exposure, skin antioxidants are severely diminished resulting in insufficient protection and cell damage.[111–113] Topical administration of antioxidants is one approach to diminish oxidative injury.[114–116] Furthermore, topical application may provide an efficient way of enriching the skin with different forms of vitamin E that have a potentially higher antioxidant activity than α-tocopherol. However, UV-irradiation depletes the various vitamin E forms similarly.[117] This suggests that the chromanol nucleus readily absorbs the UV-radiation and destroys the vitamin E.

All vitamin E forms in mouse skin, including α- and γ-tocotrienols, decreased similarly in response to UV-irradiation, demonstrating that they all afford similar antioxidant protection. The TRF application increased skin concentrations of the various vitamin E homologues, and after exposure to UV-light, all forms of vitamin E were at higher concentrations than found in vehicle-treated skin.

Ozone. Ozone is the major air pollutant in photochemical smog and the presence of ozone in the air poses a significant health concern.[118] Ozone exposure causes oxidation and peroxidation of biomolecules both directly and/or via secondary products of ozone reactions.[119–122] One of the most important mechanisms of ozone injury is peroxidation of lipids, especially unsaturated fatty acids;[120,122] *in vitro*, vitamin E appears to prevent the propagation of this reaction.[121]

Murine skin was found to be sensitive to ozone. Exposure of hairless mice to 10 p.p.m. ozone for 2 h increased skin malondialdehyde (MDA), a parameter of lipid peroxidation, but did not significantly deplete whole skin vitamin E.[123] By contrast to inherent vitamin E, skin with topically applied vitamin E was readily susceptible to ozone damage.

To evaluate where ozone caused damage to murine skin, the various layers were analyzed following exposure to 10 p.p.m. ozone.[124] Ozone only damaged the upper epidermis by depleting both vitamins C and E and increased MDA concentrations. Antioxidants in the lower layers of skin were unaffected. Assessment of the antioxidant content of the uppermost layer, the stratum corneum, was carried out using new methodologies to measure vitamin E and MDA in sequential tape strippings of mouse stratum corneum. Ozone exposures as low as 1 p.p.m. depleted vitamin E and 5 p.p.m. increased MDA formation in stratum corneum. Remarkably even 1 p.p.m. ozone for 2 h on six consecutive days significantly depleted vitamin E and increased MDA. This level of exposure is within ozone levels to which people are exposed in urban environments.[125]

These studies suggest that skin is quite susceptible to oxidative stress and that the stratum corneum serves as a barrier to oxidative insults.

Conclusion

Vitamin E is an essential nutrient. As we begin to understand the role of oxidative stress in human health, the adequacy of human vitamin E nutriture becomes a difficult question to address. It is apparent that overt vitamin E deficiency does not readily occur in humans. However, subjects with genetic defects in either vitamin E transport or in lipid transport do become vitamin E deficient and clearly should be supplemented with vitamin E. The larger question of the benefits of supplemental vitamin E to normal individuals is quite controversial. Human trials assessing whether vitamin E supplements have beneficial effects are under way and the results will be important in addressing this question.

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