Chapter 9
Vitamin E

Summary of the role of vitamin E in human metabolic processes

A large body of scientific evidence indicates that reactive free radicals are involved in many diseases, including heart disease and cancers (*1*). Cells contain many potentially oxidizable substrates such as polyunsaturated fatty acids (PUFAs), proteins, and DNA. Therefore, a complex antioxidant defence system normally protects cells from the injurious effects of endogenously produced free radicals as well as from species of exogenous origin such as cigarette smoke and pollutants. Should our exposure to free radicals exceed the protective capacity of the antioxidant defence system, a phenomenon often referred to as oxidative stress (*2*), then damage to biologic molecules may occur. There is considerable evidence that disease causes an increase in oxidative stress; therefore, consumption of foods rich in antioxidants, which are potentially able to quench or neutralise excess radicals, may play an important role in modifying the development of such diseases.

Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defence system and is exclusively obtained from the diet. The term "vitamin E" refers to a family of eight naturally occurring homologues that are synthesised by plants from homogentisic acid. All are derivatives of 6-chromanol and differ in the number and position of methyl groups on the ring structure. The four tocopherol homologues (*d*-α-, *d*-β-, *d*-γ-, and *d*-δ-) have a saturated 16-carbon phytyl side chain, whereas the tocotrienols (*d*-α-, *d*-β-, *d*-γ-, and *d*-δ-) have three double bonds on the side chain. There is also a widely available synthetic form, *dl*-α-tocopherol, prepared by coupling trimethylhydroquinone with isophytol. This consists of a mixture of eight stereoisomers in approximately equal amounts; these isomers are differentiated by rotations of the phytyl chain in various directions that do not occur naturally. For dietary purposes, vitamin E activity is expressed as α-tocopherol equivalents (α-TEs). One α-TE is the activity of 1 mg *RRR*-α-tocopherol (*d*-α-tocopherol). To estimate the α-TE of mixed diet containing natural forms of vitamin E, the number of milligrams of β-tocopherol should be multiplied by 0.5, γ-tocopherol by 0.1, and α-tocotrienol by 0.3. Any of the synthetic all-*rac*-α-tocopherol (*dl*-α-tocopherol) should be multiplied by 0.74. One milligram of the latter compound in the acetate form is equivalent to 1 IU of vitamin E.

Vitamin E is an example of a phenolic antioxidant. Such molecules readily donate the hydrogen from the hydroxyl (-OH) group on the ring structure to free radicals, which then become unreactive. On donating the hydrogen, the phenolic compound itself becomes a relatively unreactive free radical because the unpaired electron on the oxygen atom is usually delocalised into the aromatic ring structure thereby increasing its stability (*3*).

The major biologic role of vitamin E is to protect PUFAs and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals. Vitamin E is located primarily within the phospholipid bilayer of cell membranes. It is particularly effective in preventing lipid peroxidation, a series of chemical reactions involving the oxidative deterioration of PUFAs. Elevated levels of lipid peroxidation products are associated with numerous diseases and clinical conditions (*4*). Although vitamin E is primarily located in cell and organelle membranes where it can exert its maximum protective effect, its concentration may only be one molecule for every 2000 phospholipid molecules.

This suggests that after its reaction with free radicals it is rapidly regenerated, possibly by other antioxidants (5).

Absorption of vitamin E from the intestine depends on adequate pancreatic function, biliary secretion, and micelle formation. Conditions for absorption are like those for dietary lipid, that is, efficient emulsification, solubilisation within mixed bile salt micelles, uptake by enterocytes, and secretion into the circulation via the lymphatic system (*6*). Emulsification takes place initially in the stomach and then in the small intestine in the presence of pancreatic and biliary secretions. The resulting mixed micelle aggregates the vitamin E molecules, solubilises the vitamin E, and then transports it to the brush border membrane of the enterocyte probably by passive diffusion. Within the enterocyte, tocopherol is incorporated into chylomicrons and secreted into the intracellular space and lymphatic system and subsequently into the blood stream. Tocopherol esters, present in processed foods and vitamin supplements, must be hydrolysed in the small intestine before absorption.

Vitamin E is transported in the blood by the plasma lipoproteins and erythrocytes. Chylomicrons carry tocopherol from the enterocyte to the liver, where they are incorporated into parenchymal cells as chylomicron remnants. The catabolism of chylomicrons takes place in the systemic circulation through the action of cellular lipoprotein lipase. During this process tocopherol can be transferred to high-density lipoproteins (HDLs). The tocopherol in HDLs can transfer to other circulating lipoproteins, such as LDLs and very low-density lipoproteins (VLDLs) (*7*). During the conversion of VLDL to LDL in the circulation, some α-tocopherol remains within the core lipids and thus is incorporated in LDL. Most α-tocopherol then enters the cells of peripheral tissues within the intact lipoprotein through the LDL receptor pathway, although some may be taken up by membrane binding sites recognising apolipoprotein A-I and A-II present on HDL (*8*).

Although the process of absorption of all the tocopherol homologues in our diet is similar, the α form predominates in blood and tissue. This is due to the action of binding proteins that preferentially select the α form over the others. In the first instance, a 30-kDa binding protein unique to the liver cytoplasm preferentially incorporates α-tocopherol in the nascent VLDL (*9*). This form also accumulates in non-hepatic tissues, particularly at sites where free radical production is greatest, such as in the membranes of mitochondria and endoplasmic reticulum in the heart and lungs (*10*).

Hepatic intracellular transport may be expedited by a 14.2-kDa binding protein that binds α-tocopherol in preference to the other homologues (*11*). Other proteinaceous sites with apparent tocopherol-binding abilities have been found on erythrocytes, adrenal membranes, and smooth muscle cells (*12*). These may serve as vitamin E receptors which orient the molecule within the membrane for optimum antioxidant function.

These selective mechanisms explain why vitamin E homologues have markedly differing antioxidant abilities in biologic systems and illustrates the important distinction between the *in vitro* antioxidant effectiveness of a substance in the stabilisation of, for example, a food product and its *in vivo* potency as an antioxidant. From a nutritional perspective, the most important form of vitamin E is α-tocopherol; this is corroborated in animal model tests of biopotency which assess the ability of the various homologues to prevent foetal absorption and muscular dystrophies (***Table 22***).

Plasma vitamin E concentrations vary little over a wide range of dietary intakes. Even daily supplements of the order of 1600 IU/day for 3 weeks only increased plasma levels 2–3 times and on cessation of treatment plasma levels returned to pretreatment levels in 5 days (*13*). Likewise, tissue concentrations only increased by a similar amount when patients undergoing heart surgery were given 300 mg/day of the natural stereoisomer for 2 weeks

preoperatively (*14*). Kinetic studies with deuterated tocopherol (*15*) suggest that there is rapid equilibration of new tocopherol in erythrocytes, liver, and spleen but that turnover in other tissues such as heart, muscle, and adipose tissue is much slower. The brain is markedly resistant to depletion and repletion with vitamin E (*16*). This presumably reflects an adaptive mechanism to avoid detrimental oxidative reactions in this key organ.

The primary oxidation product of α-tocopherol is a tocopheryl quinone that can be conjugated to yield the glucuronate after prior reduction to the hydroquinone. This is excreted in the bile or further degraded in the kidneys to α-tocopheronic acid and hence excreted in the bile. Those vitamin E homologues not preferentially selected by the hepatic binding proteins are eliminated during the process of nascent VLDL secretion in the liver and probably excreted via the bile (*17*). Some vitamin E may also be excreted via skin sebaceous glands (*18*).

Table 22

Approximate biological activity of naturally occurring tocopherols and tocotrienols compared with *d*-α-tocopherol

Common name	Biological activity compared with *d*-α-tocopherol, %
d-α-tocopherol	100
d-β-tocopherol	50
d-γ-tocopherol	10
d-δ-tocopherol	3
d-α-tocotrienol	30
d-β-tocotrienol	5
d-γ-tocotrienol	not known
d-δ-tocotrienol	not known

Defining populations at risk of vitamin E deficiency

There are many signs of vitamin E deficiency in animals most of which are related to damage to cell membranes and leakage of cell contents to external fluids. Disorders provoked, for example, by traces of peroxidized PUFAs in the diets of animals with low vitamin E status are cardiac or skeletal myopathies, neuropathies, and liver necrosis (*19*) (***Table 23***). Muscle and neurological problems are also a consequence of human vitamin E deficiency (*20*). Early diagnostic signs of deficiency include leakage of muscle enzymes such as creatine kinase and pyruvate kinase into plasma, increased levels of lipid peroxidation products in plasma, and increased erythrocyte haemolysis.

The assessment of the vitamin E requirement for humans is confounded by the infrequent occurrence of clinical signs of deficiency because these usually only develop in adults with fat-malabsorption syndromes or liver disease, in individuals with genetic anomalies in transport or binding proteins, and possibly in premature infants (*19, 21*). This suggests that diets contain sufficient vitamin E to satisfy nutritional needs.

Several animal models (*22*) suggest that increasing intakes of vitamin E inhibit the progression of vascular disease by preventing the oxidation of LDL. Evidence suggests that oxidized lipoprotein is a key event in the development of the atheromatous plaque which may ultimately occlude the blood vessel (*23*).

Table 23

Diseases and syndromes in animals associated with vitamin E deficiency and excess intakes of polyunsaturated fatty acids

Syndrome	Affected organ or tissue	Species
Encephalomalacia	Cerebellum	Chick
Exudative diathesis	Vascular	Turkey
Microcytic anaemia	Blood, bone marrow	Chick
Macrocytic anaemia	Blood, bone marrow	Monkey
Pancreatic fibrosis	Pancreas	Chick, mouse
Liver necrosis	Liver	Pig, rat
Muscular degeneration	Skeletal muscle	Pig, rat, mouse
Microangiopathy	Heart muscle	Pig, lamb, calf
Kidney degeneration	Kidney tubules	Monkey, rat
Steatitis	Adipose tissue	Pig, chick
Testicular degeneration	Testes	Pig, calf, chick
Malignant hyperthermia	Skeletal muscle	Pig

Human studies, however, have been less consistent in providing evidence for a role of vitamin E in preventing heart disease. Vitamin E supplements reduce *ex vivo* oxidizability of plasma LDLs but there is no correlation between *ex vivo* lipoprotein oxidizability and endogenous vitamin E levels in an unsupplemented population (*24*). Likewise, the few randomised double-blind, placebo-controlled intervention trials with human volunteers which focused on the relationship between vitamin E and cardiovascular disease have given inconsistent results. There was a marked reduction in non-fatal myocardial infarction in patients with coronary artery disease (as defined by angiogram) who were randomly assigned to take pharmacologic doses of vitamin E (400 and 800 mg/day) or placebo in the Cambridge Heart Antioxidant Study involving 2000 men and women (*25*). However, the incidence of major coronary events in male smokers who received 20 mg/day of vitamin E for approximately 6 years was not reduced in the Alpha-Tocopherol, Beta-Carotene study (*26*).

Epidemiologic studies suggest that dietary vitamin E influences the risk of cardiovascular disease. Gey *et al*. (*27*) reported that lipid-standardized plasma vitamin E concentrations in middle-aged men across 16 European countries predicted 62 percent of the variance in the mortality from ischaemic heart disease. In the United States both the Nurses Health Study (*28*) involving 87000 females in an 8-year follow-up and the Health Professionals Follow-up Study in 40000 men (*29*) concluded that persons taking supplements of 100 mg/day or more of vitamin E for at least 2 years had approximately a 40 percent lower incidence of myocardial infarction and cardiovascular mortality than did those who did not use supplements. However, in US studies there was no influence of dietary vitamin E alone on incidence of cardiovascular disease when those taking supplements were removed from the analyses. A possible explanation for the significant relationship between dietary vitamin E and cardiovascular disease in European countries but not in the United States may be found in the widely differing sources of vitamin E in European countries. It is reported that sunflower seed oil, which is rich in α-tocopherol, tends to be consumed more widely in the southern European countries with the lower cardiovascular disease risk than in northern European countries where soybean oil, which contains more of the γ form, is preferred (*30*) (***Table 24***). However, a study carried out which compared plasma α- and γ-tocopherol concentrations in middle-aged men and women in Toulouse (southern France) with Belfast (Northern Ireland) found that the concentrations of γ-tocopherol in Belfast were twice as high as those in Toulouse; α-tocopherol concentrations were identical in men in both countries but higher in women in Belfast than in Toulouse ($P<0.001$) (*31*).

Table 24

Cross-country correlations between coronary heart disease mortality in men and the supply of vitamin E homologues across 24 European countries

Homologue	Correlation coefficient, *r*
Total vitamin E	-0.386
d-α-tocopherol	-0.753
d-β-tocopherol	-0.345
d-γ-tocopherol	-0.001
d-δ-tocopherol	0.098
d-α-tocotrienol	-0.072
d-β-tocotrienol	-0.329
d-γ-tocotrienol	-0.210

The correlation with *d*-α-tocopherol is highly significant ($P<0.001$) whereas all other correlations do not achieve statistical significance.
Source: Based on reference *30*.

It has also been suggested vitamin E supplementation (200–400 mg/day) may be appropriate therapeutically to moderate some aspects of degenerative diseases such as Parkinson's disease, reduce the severity of neurologic disorders such as tardive dyskinesia, prevent periventricular haemorrhage in pre-term babies, reduce tissue injury arising from ischaemia and reperfusion during surgery, delay cataract development, and improve mobility in arthritis sufferers (*32*). However, very high doses may also induce adverse pro-oxidant effects (*33*), and the long-term advantages of such treatments have not been proven.

Delineation of dietary sources and possible limitations to its availability worldwide

Because vitamin E is naturally present in plant-based diets and animal products and is often added by manufacturers to vegetable oils and processed foods, intakes are probably adequate to avoid overt deficiency in most situations. Exceptions may be during ecologic disasters and cultural conflicts resulting in food deprivation and famine.

Analysis of the Food and Agriculture Organization of the United Nations country food balance sheets indicates that about half the α-tocopherol in a typical northern European diet such as in the United Kingdom is derived from vegetable oils (*30*). Animal fats, vegetables, and meats each contribute about 10 percent to the total *per capita* supply and fruit, nuts, cereals, and dairy products each contribute about 4 percent. Less than 2 percent is each obtained from eggs, fish and pulses.

There are marked differences in *per capita* α-tocopherol supply among different countries ranging from approximately 8-10 mg/head/day (e.g., Iceland, Finland, New Zealand, and Japan) to 20–25 mg/head/day (e.g., France, Greece, and Spain) (*30*). This variation can be ascribed mainly to the type and quantity of dietary oils used in different countries and the proportion of the different homologues in the oils (***Table 25***). For example, sunflower seed oil contains approximately 55 mg α-tocopherol/100 g in contrast to soybean oil that contains only 8 mg/100 ml (*34*). Consumption of these oils varies markedly among countries. Soybean, a rich source of the less biologically active γ form, is most commonly used in northern European countries whereas sunflower seed oils, which mainly contain the α form, are generally used in southern Europe (*30*).

Table 25

Vitamin E content (mg tocopherol/100g) in vegetable oils

Oil	α–tocopherol	γ–tocopherol	δ–tocopherol	α - tocotrienol
Coconut	0.5	0	0.6	0.5
Maize (Corn)	11.2	60.2	1.8	0
Palm	25.6	31.6	7.0	14.3
Olive	5.1	Trace	0	0
Peanut	13.0	21.4	2.1	0
Soybean	10.1	59.3	26.4	0
Wheatgerm	133.0	26.0	27.1	2.6
Sunflower	48.7	5.1	0.8	0

Source: Slover HT, 1971. (34)

Summary of evidence for determining recommended nutrient intakes

In the chapter on antioxidants, it was decided that there was insufficient evidence to enable a recommended nutrient intake (RNI) to be based on the additional health benefits obtainable from nutrient intakes above those usually found in the diet. Even for vitamin E with its important biologic antioxidant properties, there was no consistent evidence for protection against chronic disease from dietary supplements. Nevertheless, the main function of vitamin E appears to be that of preventing oxidation of PUFAs, and this has been used by those bodies proposing RNIs for vitamin E because there is considerable evidence in different animal species that low vitamin E and PUFAs excess gives rise to a wide variety of clinical signs.

There is very little clinical evidence of deficiency disease in humans except in certain inherited conditions where the metabolism of vitamin E is disturbed. Even biochemical evidence of poor vitamin E status in both adults and children is minimal. Meta-analysis of data collected within European countries indicates that optimum intakes may be implied when plasma concentrations of vitamin E exceed 25–30 μmol/L of lipid-standardized α-tocopherol (*35*). However, this approach should be treated with caution, as plasma vitamin E concentrations do not necessarily reflect intakes or tissue reserves because only 1 percent of the body tocopherol may be in the blood (*36*) and the amount in the circulation is strongly influenced by circulating lipid (*37*). Nevertheless, the lipid-standardized vitamin E concentration (e.g., tocopherol-cholesterol ratio) greater that 2.25 (calculated as μmol/mmol) is believed to represent satisfactory vitamin E status (*36, 37*). The erythrocytes of subjects with values below this concentration of vitamin E may show evidence of an increasing tendency to haemolyze when exposed to oxidizing agents and thus such values should be taken as an indication of biochemical deficiency (*38*). However, the development of clinical evidence of vitamin E deficiency (e.g., muscle damage or neurologic lesions) can take several years of exposure to extremely low vitamin E levels (*39*).

The main factor used to assess the adequacy of vitamin E intakes by the US and UK advisory bodies was the dietary intake of PUFAs. PUFAs are very susceptible to oxidation, and their increased intake without a concomitant increase in vitamin E can lead to a reduction in plasma vitamin E concentrations (*40*) and to elevations in some indexes of oxidative damage in human volunteers (*41*). Generally, however, diets high in PUFAs are also high in vitamin E, and to set a dietary recommendation based on extremes of PUFA intake would deviate considerably from median intakes of vitamin E in most Western populations. Hence 'safe' allowances for the United Kingdom (men 10 and women 7 mg/day) (*42*) and 'arbitrary' allowances for the United States (men 10 and women 8 mg/day) (*43*) for vitamin E intakes approximate the median intakes in those countries. It is worth noting that there were only 11 (0.7 percent) subjects out of 1629 adults in the 1986–1987 British Nutrition Survey who had

α-tocopherol – cholesterol ratios <2.25. Furthermore, although the high intake of soybean oil with its high content of γ-tocopherol substitutes for the intake of α-tocopherol in the British diet, a comparison of α-tocopherol-cholesterol ratios found almost identical results in two groups of randomly selected, middle-aged adults in Belfast (Northern Ireland) and Toulouse (France), two countries with very different intakes of α-tocopherol (*34*) and cardiovascular risk (*31*).

It is suggested that when the main PUFA in the diet is linoleic acid, a *d*-α-tocopherol-PUFA ratio of 0.4 (expressed as mg tocopherol per g PUFA) is adequate for adult humans (*44, 45*), and the ratio has been recommended in the United Kingdom for infant formulas (*46*). Use of this ratio to calculate the vitamin E requirements of men and women with energy intakes of 2550 and 1940 kcal/day containing PUFA at 6 percent of the energy intake (approximately 17 and 13 g, respectively) (*42*) produced values of 7 and 5 mg/day of α-TEs, respectively. In both the United States and the United Kingdom, median intakes of α-TE are in excess of these amounts and the α-tocopherol-PUFA ratio is approximately 0.6 (*47*), which is well above the 0.4 ratio which would be considered adequate. The Nutrition Working Group of the International Life Sciences Institute Europe (*48*) has suggested an intake of 12 mg α-tocopherol for a daily intake of 14 g PUFAs to compensate for the high consumption of soya oil in certain countries where over 50 percent of vitamin E intake is accounted for by the less biologically active γ form. As indicated above, however, plasma concentrations in France and Northern Ireland suggest that an increased amount of dietary vitamin E is not necessary to maintain satisfactory plasma concentrations (*31*).

At present, data are not sufficient to formulate recommendations for vitamin E intake for different age groups except for infancy. There is some indication that new-born infants, particularly if born prematurely, are vulnerable to oxidative stress because of low body stores of vitamin E, impaired absorption, and reduced transport capacity resulting from low concentrations at birth of circulating low-density lipoproteins (*49*). However, term infants almost achieve adult plasma vitamin E concentrations in the first week (*50*) and although the concentration of vitamin E in early human milk can be variable, after 12 days it remains fairly constant at 0.32 mg TE/100 ml milk (51). Thus a human-milk-fed infant consuming 850 ml would have an intake of 2.7 mg. It seems reasonable that formula milk should not contain less than 0.3 mg TE/100 ml of reconstituted feed and not less than 0.4 mg TE/g PUFA.

No specific recommendations concerning the vitamin E requirements in pregnancy and lactation have been made by other advisory bodies (*42, 43*) mainly because there is no evidence of vitamin E requirements different from those of other adults and presumably also as the increased energy intake would compensate for the increased needs for infant growth and milk synthesis.

Vitamin E appears to have very low toxicity, and amounts of 100–200 mg of the synthetic all-*rac*-α-tocopherol are consumed widely as supplements (*28, 29*). Evidence of pro-oxidant damage has been associated with the feeding of supplements but usually only at very high doses (e.g., >1000 mg/day) (*33*).

Future research

More investigation is required of the role of vitamin E in biologic processes which do not necessarily involve its antioxidant function. These processes include:

- structural roles in the maintenance of cell membrane integrity;
- anti-inflammatory effects by direct and regulatory interaction with the prostaglandin synthetase complex of enzymes, which participate in the metabolism of arachidonic acid;
- DNA synthesis;

- stimulation of the immune response; and
- regulation of intercellular signalling and cell proliferation through modulation of protein kinase-C.

Similarly, more investigation is required of the growing evidence that inadequate vitamin E status may increase susceptibility to infection particularly by allowing the genomes of certain relatively benign viruses to convert to more virulent strains (*52*).

There is an important need to define optimum vitamin E intakes. Intervention trials with morbidity and mortality endpoints may take years to complete. One approach to circumvent this delay may be to assess the effects of different intakes of vitamin E on biomarkers of oxidative damage to lipids, proteins, and DNA because their occurrence *in vivo* is implicated in many diseases, including vascular disease and certain cancers.

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