Optimal nutrition: vitamin E

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Interest in the role of vitamin E in disease prevention has encouraged the search for reliable indices of vitamin E status. Most studies in human subjects make use of static markers, usually α-tocopherol concentrations in plasma or serum. Plasma or serum α-tocopherol concentrations of < 11·6, 11·6–16·2, and > 16·2 μmol/l are normally regarded as indicating deficient, low and acceptable vitamin E status respectively, although more recently it has been suggested that the optimal plasma α-tocopherol concentration for protection against cardiovascular disease and cancer is > 30 μmol/l at common plasma lipid concentrations in combination with plasma vitamin C concentrations of > 50 μmol/l and > 0·4 μmol β-carotene/l. Assessment of vitamin E status has also been based on α-tocopherol concentrations in erythrocytes, lymphocytes, platelets, lipoproteins, adipose tissue, buccal mucosal cells and LDL, and on α- tocopherol : γ-tocopherol in serum or plasma. Erythrocyte susceptibility to haemolysis or lipid oxidation, breath hydrocarbon exhalation, oxidative resistance of LDL, and α-tocopheryl quinone concentrations in cerebrospinal fluid have been used as functional markers of vitamin E status. However, many of these tests tend to be non-specific and poorly standardized. The recognition that vitamin E has important roles in platelet, vascular and immune function in addition to its antioxidant properties may lead to the identification of more specific biomarkers of vitamin E status.

Vitamin E: Biomarkers

Vitamin E is the generic term used to describe at least eight naturally-occurring compounds that exhibit the biological activity of α-tocopherol. The group comprises α-, β-, γ- and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol. All these compounds occur as a variety of isomers. *RRR*-α-tocopherol has the highest biological activity according to rat fetal resorption (Weiser & Vecchi, 1982) or pyruvate kinase (*EC* 2.7.1.40; Machlin *et al*. 1982) assays, and accounts for approximately 90 % of the vitamin E activity found in tissues (Cohn, 1997). The other tocopherols and tocotrienols are less biologically active but they are at least as abundant as α-tocopherol in certain foods (notably γ-tocopherol in maize oil, γ- and δ-tocopherol in soyabean oil and γ-tocopherol and α-tocotrienol in palm oil; Sheppard *et al*. 1993). The commercially-available synthetic forms of vitamin E comprise an approximately equal mixture of eight stereoisomeric forms of α-tocopherol. The expression of vitamin E activity has changed from the former US Pharmacopeia vitamin E units (IU) to mg α-tocopherol equivalents (α-TE), where 1 mg α-TE is equal to the activity of 1 mg *RRR*-α-tocopherol or 1·49 mg all-*rac*-α-tocopheryl acetate.

The most widely accepted biological function of vitamin E is its antioxidant property. In addition, it directly influences cellular responses to oxidative stress through modulation of signal-transduction pathways (Azzi *et al*. 1992). Increasing evidence implicates free radical-mediated cell and tissue damage in the pathogenesis of various degenerative diseases, including cardiovascular disease, cancer, inflammatory diseases, diabetes mellitus, neuro-degenerative diseases and cataract. Vitamin E is the most effective chain-breaking lipid-soluble antioxidant in biological membranes, where it contributes to membrane stability and protects critical cellular structures against damage from free radicals and reactive products of lipid oxidation (Meydani, 1995). Until clinical trials involving supplements of vitamin E and other antioxidant micro-nutrients were started in the mid-1980s, prospective studies involving the examination of serum vitamin E levels and the occurrence of cardiovascular disease and cancer were the primary source of evidence, and results from these types of studies are inconsistent regarding possible disease prevention by vitamin E (Rock *et al*. 1996). The evidence over the last 10 years is supportive of a role for vitamin E in the

Abbreviations: oxo8dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; α-TE, α-tocopherol equivalents.

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prevention of cardiovascular disease, but less so in the prevention of cancer. However, the results of several ongoing large randomized intervention trials must be awaited before conclusions can be drawn.

Biomarkers in nutritional status assessment

The intense interest in the role of vitamin E in disease prevention has encouraged the search for reliable indices of vitamin E status for use in basic research, epidemiology, clinical trials and large-scale intervention studies. In principle, nutritional status can be assessed by measuring dietary intake, by clinical examination, or by the measurement of biochemical and/or physiological markers. The reliability of dietary intake data is reduced by factors such as incomplete food–nutrient databases, variability in food composition or preparation, inconsistency of subjects' eating habits, recall biases between cases and controls, and inter-individual differences in nutrient absorption and metabolism. Also, high intakes of a nutrient might only be a marker for some other nutrient, non-nutrient, dietary practice or lifestyle habit that could itself be the true preventive factor (Blumberg, 1995). Clinical methods lack the sensitivity to diagnose marginal status, in which the depletion of body stores is serious enough to cause functional impairment but not clinical signs. Indeed, for some nutrients, including vitamin E, clinical deficiency is rare. Thus, increasing emphasis is being placed on the identification, validation and exploitation of more sensitive biochemical or physiological markers (biomarkers) of nutrient status. In parallel with these investigations is the development of valid early and intermediate markers of disease risk and disease progression. Taken together, these markers provide information on the bioavailability, turnover, metabolism, interactions, and mechanisms of action of nutrients which is critical for identifying the types of populations that would be most responsive in large clinical trials, the optimal timing of the intervention, and optimal trial duration and design. Appropriate markers can also be used to validate dietary intake data and to obtain additional information from banked specimens (Kohlmeier, 1995).

Biomarkers of nutrient status may be referred to as 'static' or 'functional'. Examples of static markers include the concentrations of a nutrient or its metabolites in blood or urine, while functional markers include the level or response of a nutrient-related variable such as an enzyme activity, an abnormal metabolite (which might also be a marker of disease risk) or a physiological function. Ideally, the biomarkers selected should be inexpensive to collect and analyse, requiring small amounts of specimen obtained in a low- to non-invasive manner. In the case of biomarkers of dietary exposure, they should persist for long periods of time, represent the integrated exposure over time and all routes of entry, and be specific and highly predictive of that exposure; assays for the markers should be sensitive, specific and reliable, and baseline values in the unexposed population should be low (Kohlmeier, 1995). However, these requirements are seldom, if ever, completely met.

Biomarkers of vitamin E status

Static markers

Most studies measuring vitamin E status in human subjects make use of static markers, usually α-tocopherol concentrations in plasma or serum. α-Tocopherol may also be measured in erythrocytes, lymphocytes, platelets, lipoproteins, adipose tissue and buccal mucosal cells. γ-Tocopherol may also be measured. One of the questions to be considered when using these less common biomarkers is whether they are markers of the dietary intake or of the internal dose. To answer this question, a better knowledge of vitamin E metabolism and distribution is required.

Plasma or serum α-tocopherol concentration

This variable remains the most used biomarker to assess vitamin E status and, consequently, is the one for which most data are available (Farrell *et al*. 1978; Looker *et al*. 1989; Hercberg *et al*. 1994; Winklhofer-Roob *et al*. 1997). The measurement is technically simple, but there are a variety of confounding factors, including the effects of age, sex, plasma lipids, lipid-lowering drugs and smoking, that must be taken into consideration (Berr *et al*. 1998). In addition, many disease states are associated with changes in vitamin E status, and it is not easy to determine whether these changes are a cause or a consequence of the disease process. Plasma or serum α-tocopherol concentrations of $< 11{\cdot}6$, $11{\cdot}6$–$16{\cdot}2$, and $> 16{\cdot}2$ μmol/l are normally regarded as indicating deficient, low and acceptable vitamin E status respectively (Sauberlich *et al*. 1974). However, based on a review of evidence from cross-cultural and prospective epidemiology and case–control studies, Gey (1995*a*,*b*) contended that the optimal plasma α-tocopherol concentration for protection against cardiovascular disease and cancer was > 30 μmol/l at common plasma lipid concentrations (cholesterol 2200 mg/l and triacylglycerols 1000 mg/l) in combination with plasma vitamin C concentrations of > 50 μmol/l and $> 0{\cdot}4$ μmol β-carotene/l ($> 0{\cdot}5$ μmol total carotene/l). Traber & Sies (1996) estimated that, on average, a daily dietary intake of about 15–30 mg α-tocopherol is required to maintain this plasma level, an amount which could be obtained from dietary sources if a concerted effort was made to eat foods high in vitamin E. In contrast, the amounts of supplemental vitamin E suggested as protective from epidemiological studies are many times higher than those that could be obtained from the diet.

The correlation between α-tocopherol and blood lipids, especially cholesterol, is very strong, particularly in hypo- or hyperlipaemia. Consequently, it is recommended that plasma or serum α-tocopherol concentrations be lipid-corrected (i.e. expressed relative to either the sum of cholesterol and triacylglycerols or to cholesterol alone). For convenience, α-tocopherol : cholesterol is the simplest to obtain and probably the most useful, with values below $2{\cdot}2$ μmol α-tocopherol/mmol cholesterol indicating a risk of deficiency (Thurnham *et al*. 1986). The optimal value for α-tocopherol : cholesterol was estimated by Gey (1995*a*,*b*) to be $> 5{\cdot}2$ μmol/mmol.

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α-Tocopherol in other blood components

Erythrocyte α-tocopherol concentrations have been used in attempting to evaluate vitamin E status in a variety of subject groups such as cystic fibrosis patients (Farrell *et al.* 1977), premature infants and children (Mino *et al.* 1985), and hypertensive patients (Wen *et al.* 1996). The latter authors reported that erythrocyte α-tocopherol concentrations were significantly lower in a group of hypertensive patients compared with normotensive controls, although plasma α-tocopherol levels were similar in both groups. A disadvantage of erythrocyte α-tocopherol is that values are affected by packed cell volume and also by plasma lipid levels (Bieri *et al.* 1977).

It has been suggested that α-tocopherol concentrations in platelets and lymphocytes may be more useful biomarkers of vitamin E status than those in erythrocytes. Lehmann *et al.* (1988) measured α-tocopherol concentrations in plasma, plasma lipids, erythrocytes, platelets and lymphocytes from twenty subjects on graded intakes of vitamin E. α-Tocopherol levels were determined before supplementation, following 6 weeks of supplementation with 30 mg DL-α-tocopheryl acetate/d, and after supplementation with 100 mg DL-α-tocopheryl acetate/d for a further 6 weeks. Platelets were more sensitive to changes in vitamin E intake than the other blood elements. In contrast, Kaempf *et al.* (1994) assessed the vitamin E status of twenty-eight healthy newborn infants and ninety-two children aged from 5 months to 16 years on the basis of α-tocopherol levels in plasma, erythrocytes, platelets, and mononuclear and polymorphonuclear leucocytes. They also measured α-tocopherol in buccal mucosal cells, which they suggested might reflect body stores of vitamin E. Plasma α-tocopherol levels rose significantly in neonates during the first week of life, and these changes were also observed to a slightly lesser extent in mononuclear leucocytes and buccal mucosal cells. However, erythrocyte, platelet, and polymorphonuclear leucocyte α-tocopherol levels did not change significantly during this period. In infants, young and older children, α-tocopherol levels in plasma, platelets, buccal mucosal cells and mononuclear leucocytes appeared to follow the same pattern over time, whereas those in erythrocytes and polymorphonuclear leucocytes did not. These authors proposed that α-tocopherol levels in cells with LDL receptors, such as mononuclear leucocytes and buccal mucosal cells, may be more useful markers of vitamin E status than those in erythrocytes or platelets.

Plasma or serum α-tocopherol : γ-tocopherol

Large doses of α-tocopherol displace γ-tocopherol from plasma to bile (Handelman *et al.* 1985; Baker *et al.* 1986), suggesting that α-tocopherol : γ-tocopherol in plasma or serum could be an index of α-tocopherol ingestion (e.g. from supplements) and therefore, might be useful for monitoring compliance during clinical trials. However, this possibility requires validation.

In a recent case–control study, Ohrvall *et al.* (1996) evaluated the predictive power of the serum α-tocopherol : γ-tocopherol value to discriminate between subjects with and without CHD. Serum α-tocopherol did not differ significantly between patients and healthy age-matched controls, but patients had a lower mean serum γ-tocopherol concentration and an elevated α-tocopherol : γ-tocopherol. The reasons for this finding, and its practical significance, are unclear.

γ-Tocopherol is probably worthy of much more consideration in its own right than it has received up to now. Recent studies (Christen *et al.* 1997) on the effects of α- and γ-tocopherol against peroxynitrite in LDL suggest that γ-tocopherol can act as a trap for membrane-soluble electrophilic nitrogen oxides and other electrophilic mutagens, forming stable carbon-centred adducts through its nucleophilic 5-position, which is blocked in α-tocopherol. Other studies (Clement & Bourre, 1997) demonstrated that when supplemental γ-tocopherol was supplied continuously in rat diets it induced a marked increase in α-tocopherol concentrations in serum and tissues, suggesting that there is a relationship between α- and γ-tocopherol *in vivo* and that the biopotency of dietary α-tocopherol could depend to some extent on habitual intakes of γ-tocopherol. Whether this situation is due to increased expression of hepatic α-tocopherol transfer protein mRNA, as has been shown for δ-tocopherol (Fechner *et al.* 1998), is unclear. A role for γ-tocopherol in reducing peroxides and mutagens in faeces has also been postulated (Pappas, 1996). Similarly, more consideration should be given to intakes of tocotrienols and their biological effects. These compounds have been reported to have hypocholesterolaemic properties, to be better antioxidants than the corresponding tocopherols under a variety of oxidizing conditions, and to have anti-aggregatory and anti-thrombotic effects (Qureshi & Qureshi, 1993; Serbinova *et al.* 1993).

α-Tocopherol concentration in LDL

The question of whether plasma α-tocopherol is predictive of its concentration in LDL is important in the context of the epidemiological evidence of an inverse relationship between intakes or plasma levels of vitamin E and the risk of atherosclerosis, and of the LDL oxidation hypothesis. There are considerable variations in LDL-α-tocopherol concentration between individuals and these differences were generally thought to be due mainly to differences in plasma levels. The fact that taking a vitamin E supplement leads to an increase in plasma α-tocopherol and a parallel and more or less linear increase in LDL-α-tocopherol led Jialal & Grundy (1992) to conclude that plasma levels of α-tocopherol are a practical surrogate for LDL-α-tocopherol. Jialal *et al.* (1995) observed a dose-dependent increase in plasma and lipid-standardized α-tocopherol levels and LDL-α-tocopherol with increasing dose of α-tocopherol supplementation (40, 134, 269, 537 and 805 mg α-TE for 8 weeks). However, in normal unsupplemented individuals, only LDL-γ-tocopherol was strongly correlated with plasma γ-tocopherol (r^2 0·68), while LDL-α-tocopherol concentration did not reflect plasma α-tocopherol (r^2 0·001); Ziouzenkova *et al.* 1996). This finding suggests that the α-tocopherol content of LDL may not be the exclusive determinant for the inverse relationship between plasma α-tocopherol and the risk of cardiovascular disease, and that other effects such as decreased platelet

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adhesion and aggregation, smooth muscle cell proliferation and monocyte adhesion could be major mechanisms. In any event, these results indicate that plasma α-tocopherol concentrations do not provide reliable information on α-tocopherol concentrations in LDL in unsupplemented individuals.

The value of LDL-α-tocopherol as a predictor of the severity of coronary artery disease was recently evaluated by Regnstrom *et al*. (1996) using a scoring system in which coronary angiograms were analysed for the number and size of distinct stenotic lesions (global stenosis score). Lipid-adjusted serum and LDL-α-tocopherol concentrations were significantly lower in patients (sixty-four male survivors of myocardial infarction < 45 years) than in thirty-five age-matched controls, and a significant inverse correlation was observed between LDL-vitamin E concentration, whether adjusted to lipid (r −0·477, $P < 0·001$) or protein (r −0·375, $P < 0·01$), and global coronary stenosis score.

α-Tocopherol concentrations in other tissues

Adipose tissue, liver and muscle represent the major stores of vitamin E in the body, with about 90 % of the vitamin being contained in the adipose tissue (Traber & Kayden, 1987). Although it has a very slow turnover (Bjørneboe *et al*. 1990), adipose tissue vitamin E is strongly associated with dietary intake (Schäfer & Overvad, 1990). This relationship, coupled with the fact that plasma α-tocopherol would probably be affected by an acute myocardial infarction, led to the selection of gluteal adipose tissue α-tocopherol as a long-term measure of vitamin E status in the EURAMIC study (Kardinaal *et al*. 1993) in preference to plasma α-tocopherol. Dietary fatty acids had previously been reported to be potential confounders of adipose tissue vitamin E, since vitamin E intake is positively associated with that of linoleic acid, while linoleic acid, in adipose tissue is inversely related to the risk of myocardial infarction (Wood *et al*. 1987). However, in multiple regression analysis, adjusting for age and centre waist circumference was the only independant predictor of adipose tissue α-tocopherol level in men, while in women no predictors of α-tocopherol level were found (Virtanen *et al*. 1996). There were no significant differences in adipose tissue α-tocopherol concentrations between cases of breast cancer and controls. Zhu *et al*. (1996) examined the dietary intake and breast adipose tissue concentrations of vitamin E in women diagnosed with benign breast disease and with breast cancer. Vitamin E concentrations in breast adipose tissue were significantly positively correlated with dietary vitamin E intake ($P < 0·05$). In post-menopausal women, significantly lower dietary vitamin E intake ($P < 0·01$) and breast adipose tissue vitamin E concentrations ($P < 0·05$) were observed in patients with breast cancer than in subjects with benign breast disease. One obvious limitation of adipose tissue biopsy sampling is that it is more invasive than blood sampling, and is not practical except in a research setting. However, as described earlier, buccal mucosal cells may be sampled easily and non-invasively by gentle scraping with a spatula, and are suitable for vitamin E determinations (Kaempf *et al*. 1994). It has been reported that these cells have a similar fatty acid composition to that of adipose tissue and that they reflect the daily intake (McMurchie *et al*. 1984). Further studies on this potential biomarker of body vitamin E stores are warranted.

Functional markers

Since vitamin E is the major lipid-soluble chain-breaking antioxidant in plasma and tissues, many tests have been developed which attempt to measure functional vitamin E status on the basis of the degree of lipid oxidation *in vivo*, or the resistance of cells and lipoproteins etc., to stimulated lipid oxidation *in vitro*. Many of these methods are poorly standardized and, apart from LDL oxidative susceptibility or resistance, they have had fairly limited application. Examples of tests which have been used to assess vitamin E status based on *in vivo* lipid oxidation or *in vitro* oxidative stress susceptibility in various cells, tissues and other matrices are given in the following sections.

Susceptibility of erythrocytes to haemolysis or lipid oxidation

Erythrocyte haemolysis *in vitro* stimulated by dilute H_2O_2 has been used as a functional test of vitamin E deficiency (Machlin, 1984; Miyake *et al*. 1991). The latter authors demonstrated that neonatal erythrocyte membranes have a high peroxidative potential due to their high content of polyunsaturated fatty acids, reflecting an increased requirement for vitamin E. More recently, Girodon *et al*. (1997) employed a modified erythrocyte haemolysis test using the free-radical generator 2,2′-azo-bis (2-amidinopropane) dihydrochloride to assess the effects of antioxidant vitamin and mineral supplements on oxidative stress susceptibility and oxidant defences in elderly subjects. Serum α-tocopherol : cholesterol was positively correlated ($P = 0·06$) with erythrocyte resistance to haemolysis. Although haemolysis tests reflect impaired integrity of erythrocyte membranes, they are of limited usefulness for vitamin E status assessment because of their lack of specificity, as well as the variability of test results and lack of standardization between different laboratories (Anonymous, 1988).

Malondialdehyde production during exposure of erythrocytes to H_2O_2 *in vitro* has also been used to assess vitamin E status (Cynamon & Isenberg, 1987). In a study involving children with cholestatic liver disease at risk of vitamin E deficiency and vitamin E-sufficient controls, the test was capable of detecting vitamin E deficiency which was confirmed by low plasma α-tocopherol and lipid-corrected plasma α-tocopherol concentrations. In contrast, the erythrocyte haemolysis test underestimated the prevalance of vitamin E deficiency. A limitation of the test is that malondialdehyde is estimated by measuring thiobarbituric acid-reactive substances which are not specific for malondialdehyde. For this reason the assay cannot be recommended for routine assessment of vitamin E status.

The vitamin E status of pregnant women, neonates and normal adults was assessed by measuring O_2 consumption rates in erythrocyte ghost membranes during exposure to 2,2′-azo-bis (2-amidinopropane) dihydrochloride (Mino, 1993). Despite having similar tocopherol concentrations, O_2

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consumption in ghost membranes of neonates was significantly greater ($P < 0{\cdot}05$), and in those of pregnant women slightly greater than in those from normal adults. The effect appeared to be due to differences in membrane arachidonic acid and 'active hydrogen' content. This finding suggests that the functional vitamin E status of neonates and pregnant women is poorer than that in normal adults.

Breath hydrocarbons

Lipid peroxidation results in the production of hydrocarbon gases such as pentane (from *n*-6 polyunsaturated fatty acid hydroperoxides) and ethane (from *n*-3 polyunsaturated fatty acid hydroperoxides). Both gases pass through the lungs into the expired air. Pentane and ethane levels in breath samples were correlated with *in vivo* lipid peroxidation in rats deprived of dietary vitamin E (Dillard *et al*. 1977), and pentane was inversely proportional to log vitamin E concentration in the diet (Downey *et al*. 1978). A method for measuring breath pentane exhalation in adults was described by Lemoyne *et al*. (1987). Pentane exhalation was significantly higher in vitamin E-deficient patients receiving home parenteral nutrition than in normal adults. Furthermore, breath pentane exhalation in normal subjects was significantly decreased after supplementation with vitamin E (67 mg α-TE/d for 10 d). These authors concluded that breath pentane output is a sensitive non-invasive functional test for assessing vitamin E status. However, it does not appear to have been widely applied, perhaps because of its technical demands.

Oxidative resistance of LDL

Oxidatively-modified LDL shows *in vitro* properties that could explain several phenomena in the chain of events leading to the development of atherosclerosis (Steinberg *et al*. 1989). LDL is rich in polyunsaturated fatty acids which are protected against oxidation by a range of lipophilic compounds, including α- and γ-tocopherol, β-carotene and other carotenoids, phytofluene and ubiquinol-10. When LDL is exposed to an oxidative stress these antioxidants are consumed, after which the propagation phase of lipid oxidation commences. The lag-time in the formation of conjugated dienes provides a sensitive measure of the resistance of LDL to oxidation and is widely assumed to be an indicator of atherogenic risk. Recent studies, however, have cast some doubt on this assumption. Kleinveld *et al*. (1994) demonstrated that vitamin E supplementation of hyperlipidaemic rabbits significantly ($P < 0{\cdot}001$) reduced the lag-time of LDL oxidation but did not significantly reduce the area of aorta covered by plaque compared with controls, while Freubis *et al*. (1997) reported that lag-times in LDL-receptor-deficient rabbits were not correlated with the anti-atherogenic efficacy of different antioxidants, including vitamin E.

Since the major antioxidant in LDL is α-tocopherol, it had been thought that the duration of the lag phase of LDL oxidation or the rate of oxidation during the propagation phase might be a useful indicator of the vitamin E status of an individual's LDL *in vivo*. Certainly, the oxidative resistance of LDL is increased in vitamin E-supplemented individuals, especially at intakes ≥ 269–336 mg α-TE (Jialal & Grundy, 1992; Esterbauer *et al*. 1993; Simons *et al*. 1996; Devaraj *et al*. 1997), and there are strong correlations between oxidative resistance and LDL-α-tocopherol concentration in these subjects (Jialal & Grundy, 1992; Esterbauer *et al*. 1993; Jialal *et al*. 1995). Princen *et al*. (1995) observed in vitamin E-supplemented men and women that the rate of oxidation was significantly ($P < 0{\cdot}01$) decreased at 269 and 537 mg α-TE/d, and concluded that vitamin E was the most important variable that determined oxidative resistance of LDL in the study group. However, the relationship becomes weaker in unsupplemented subjects. Frei & Gaziano (1993) observed that only 18 % of the lag phase of lipid oxidation in Cu^{2+}-incubated LDL was determined by the vitamin E (α- + γ-tocopherol) : cholesterol value of LDL in healthy subjects not taking vitamin E supplements, while others (Dieber-Rotheneder *et al*. 1991) reported no significant correlation between oxidative resistance of LDL and LDL-α-tocopherol concentration. Furthermore, a recent case–control study on patients with and without coronary artery disease demonstrated that duration of the lag phase of LDL oxidation was independent not only of dietary, plasma or LDL-vitamin E, but also of a wide range of other pro-oxidative or anti-oxidative plasma factors and coronary risk factors (Halevy *et al*. 1997). These authors speculated that a short lag phase of LDL might be an independent risk factor for coronary artery disease.

α-Tocopheryl quinone and α-tocopheryl hydroquinone

α-Tocopheryl quinone is formed *in vivo* at sites of oxidative stress, and it can be reduced enzymically to the hydroquinone (Siegel *et al*. 1997). It has been suggested that levels of α-tocopheryl quinone in cerebrospinal fluid may be an indicator of increased peroxidation in brain and an early indicator of degenerative brain diseases. Toghi *et al*. (1994) reported significantly higher concentrations of α-tocopheryl quinone in cerebrospinal fluid from Binswanger-type dementia patients, but not in patients with Alzheimer-type dementia. α-Tocopheryl hydroquinone, on the other hand, may be a previously unrecognized natural antioxidant. In studies on LDL oxidation, Neuzil *et al*. (1997) demonstrated that α-tocopheryl hydroquinone was extremely efficient at protecting ubiquinol-10, α-tocopherol and both surface and core lipids in LDL against several different oxidizing systems.

Potential new functional biomarkers of vitamin E status

There is increasing evidence that vitamin E has many other important roles in addition to protecting against lipid oxidation in biological membranes. Properties of vitamin E relating to immunostimulation, protection against DNA damage, control of smooth muscle cell proliferation, and inhibition of platelet aggregation and adhesion continue to be elucidated. In time our understanding of these properties will lead to the identification of more sensitive and disease-specific biomarkers for vitamin E. Some of these properties and potential new biomarkers are highlighted later (pp. 464–465). It should be bourne in mind that the response of most,

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if not all, these markers is likely to depend on interactions between vitamin E and many other endogenous and exogenous compounds. They should be regarded, therefore, as indices of a process rather than simply as markers of vitamin E concentration in a particular cell or tissue.

Platelet function

The transformation of flowing blood into a clot depends on the participation of platelets (Richardson & Steiner, 1993). Their adherence to exposed collagen initiates a sequence of events that begins with platelet activation and ends with the change of soluble fibrinogen to insoluble fibrin. In recent years, it has become clear that vitamin E supplementation is capable of reducing platelet adhesion (Richardson & Steiner, 1993). In healthy non-smoking normal individuals, supplementation with vitamin E over a 2-week period resulted in a significant dose-dependent decrease in platelet adhesion up to an intake level of 267 mg α-TE/d.

Platelet aggregation is also inhibited by vitamin E. Freedman *et al*. (1996) reported that in fifteen normal subjects, oral supplementation with α-tocopherol (267–805 mg α-TE/d) resulted in an increase in platelet α-tocopherol content that correlated with a marked inhibition of platelet aggregation. Supplementation of hypercholesterolaemic patients with vitamin E (267 mg α-TE/d for 6 weeks) resulted in a significant inhibition of thrombin-induced aggregation (Williams *et al*. 1997). After 6 weeks supplementation, the mean concentration producing 50 % maximum effect for thrombin-induced aggregation increased 132 % ($P < 0{\cdot}05$). Calzada *et al*. (1997) performed a double-blind randomized placebo-controlled trial on platelet function in forty healthy volunteers supplemented daily with vitamin E (300 mg), vitamin C (250 mg) or β-carotene (15 mg) for 8 weeks. Platelet function was significantly decreased by vitamin E as revealed by decreased platelet aggregation in response to ADP and arachidonic acid, increased sensitivity to inhibition by prostaglandin E, decreased plasma β-thromboglobulin concentration and decreased ADP secretion.

The studies cited previously made use of supplements far in excess of the recommended dietary allowance, and it is not clear what effects, if any, the typical range of dietary vitamin E intakes might have on platelet function. However, some authors (Williams *et al*. 1997) are of the opinion that the effects of vitamin E on platelet function could explain in part its anti-atherogenic properties.

Vascular function

In addition to their protective effects within the LDL particle, antioxidants may act at the level of the vascular cell by limiting cellular production of reactive oxygen species and, thus, cell-mediated LDL oxidation (Gokce & Frei, 1996). Furthermore, there is increasing evidence that antioxidants can also protect against vascular cell dysfunction that would otherwise promote atherogenesis, such as increased adhesion molecule expression and monocyte recruitment, impaired production or release of NO, and proliferation of smooth muscle cells. Ozer *et al*. (1995) reported that α-tocopherol activates the release of transforming growth factor-β, which is secreted by smooth muscle cells as a growth inhibitor. In contrast, LDL decreases the release of transforming growth factor-β from smooth muscle cells, thus activating growth. This finding could explain the important roles of LDL and vitamin E in increasing and decreasing the risk of atherosclerosis respectively. Devaraj *et al*. (1996) observed that interleukin 1-β secretion and monocyte–endothelial adhesion was significantly reduced by supplementation with vitamin E (805 mg α-TE/d for 8 weeks). Azzi *et al*. (1997) recently reported that α-tocopherol activates protein phosphatase 2A in aortic smooth muscle of cholesterol-fed rabbits, resulting in dephosphorylation and inhibition of protein kinase C-α activity and, ultimately, inhibition of smooth muscle cell proliferation.

Oxidative damage to DNA and DNA repair

The use of biomarkers of DNA damage and DNA repair in cancer research is well established, and several recent studies have attempted to relate vitamin E intake or status to one or more of these markers. Wang *et al*. (1996) conducted a crossover trial on seven subjects who took β-carotene (31·4 mg/d) and α-tocopheryl acetate (483 mg α-TE/d) supplements for 3 months, and reported that there was a significant inverse relationship between erythrocyte spermine (a candidate biomarker for hyperproliferation) and plasma α-tocopherol. Cadenas *et al*. (1997) fed guinea-pigs on a diet containing 600 mg vitamin C/kg and three different amounts of vitamin E (15, 150 or 1500 mg/kg diet, representing a marginal deficiency, an optimal diet and a megadose respectively) for 5 weeks, and measured liver 8-oxo-7,8-dihydro-2′-deoxyguanosine (oxo8dG), a repair product of oxidative DNA damage. Despite very large variations in the vitamin E content of the diets and liver, there were no significant differences in oxo8dG levels between groups. Similarly, Prieme *et al*. (1997) reported that supplementation of smokers with antioxidants (vitamin E, vitamin C and abiquinol-10, in a variety of combinations) for 2 months did not result in significant changes in the urinary excretion rate of oxo8dG, despite substantial changes in plasma antioxidant concentration. Sister chromatid exchanges in peripheral lymphocytes were not correlated with plasma antioxidants, including vitamin E, in smokers (van Poppel *et al*. 1993), nor was plasma α-tocopherol significantly predictive of the levels of DNA adducts in lymphocytes of current or non-smokers (Wang *et al*. 1997).

Immune system function

Vitamin E plays an important role in the maintenance of the immune system. Vitamin E deficiency impairs the immune response, while supplementation with higher than recommended dietary levels of vitamin E has, in some instances, improved immunity. Meydani *et al*. (1997) supplemented healthy elderly subjects with placebo, 44, 147, or 588 mg α-TE/d for 235 d using a double-blind randomized design. All three vitamin E doses significantly enhanced ($P < 0{\cdot}05$) delayed-type hypersensitivity score, with subjects consuming 147 mg α-TE/d having the highest percentage increase. A significantly greater ($P < 0{\cdot}05$) antibody response to

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hepatitis B was observed in subjects consuming 147 or 588 mg α-TE/d, but not in those consuming 44 mg α-TE/d. Those subjects consuming 144 mg α-TE/d also had a significant increase ($P < 0{\cdot}05$) in antibody response to tetanus toxoid vaccine. These authors concluded that 144 mg α-TE/d represented the optimal level of vitamin E for the immune response and suggested that there may be a threshold below which the immunostimulation by vitamin E does not occur. This suggestion would appear to be supported by the finding of de Waart *et al*. (1997) that vitamin E (67 mg α-TE/d for 3 months) had no beneficial effect on cellular or humoral immune responses in elderly subjects. Beharka *et al*. (1997) provided protocols for measuring a range of immunological responses, including delayed-type hypersensitivity, lymphocyte proliferation, interleukin 2 production, cytokine production of interleukin 6 and tumour necrosis factor-α, and phagocytosis, which are sensitive to changes in the availability of vitamin E and which may reflect the vitamin E status of an individual more accurately than conventional methods.

The traditional view of the relationship between nutrition and immunity has been that malnutrition interferes with the body's physical barriers or immune responses and renders the host more vulnerable to infection. However, recent work by Beck and colleagues, summarized in Beck (1997), has demonstrated that nutrition not only affects the host but can affect the pathogen as well. An avirulent coxsackievirus became virulent as a result of replicating in vitamin E- or Se-deficient mice. The conversion to virulence was due to a change in the genotype of the benign virus. The change was stable and its pathological consequences (cardiomyopathy) could be expressed even in mice of normal vitamin E or Se status. It is not known whether other nutritional deficiencies in the host might have similar effects, or whether other viruses apart from coxsackie are affected by host nutriture. However, the results suggest that promoting the concept of optimal nutrition may be important not just from the standpoint of preventing deficiency symptoms and chronic disease, but also in minimizing the evolution and spread of infectious diseases.

Conclusion

Recommended dietary allowances are designed so that, if met at a population level, almost all individuals would meet their requirements and avoid clinical deficiency symptoms. Nowadays, however, there is much greater interest, at least in the developed world, in the possibility that intakes of specific nutrients at higher levels than the recommended dietary allowance may confer health benefits over and above the prevention of deficiency symptoms. A consensus about the exact daily intake of vitamin E for optimal health protection has not yet been reached. Some authors believe that the scientific evidence is strong enough already, especially for cardiovascular disease, to recommend daily intakes of the order of 87–100 mg α-TE/d or more (Horwitt, 1991; Packer, 1993; Weber *et al*. 1997). Realistically, these levels could only be achieved at present by taking supplements. However, other workers believe that vitamin E supplementation should not be endorsed and the focus should remain on eating a healthy diet, rich in fruit and vegetables, until conclusive evidence of benefits of vitamin E is demonstrated in clinical trials (Rexrode & Manson, 1996). In the meantime, efforts should continue to try to elucidate the precise biological mechanisms of α-tocopherol at the cellular and subcellular level, both as an antioxidant and as a modulator of platelet, vascular and immune function. The other tocopherols and tocotrienols should also be studied more carefully, as in some cases they seem to have properties not shared by α-tocopherol. The identification, validation and incorporation into study design of better markers of vitamin E status and function, and of reliable markers of disease risk and progression, will greatly assist our understanding of this important nutrient.

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