

EXPERT GROUP ON VITAMINS AND MINERALS

REVIEW OF VITAMIN E – REVISED VERSION

The attached review of vitamin E is slightly amended version of the revised paper considered at the meetings in July 2000 and October 2001. New information on large scale clinical trials has been included.

The following annexes are also included:

- Annex 1 Tables referred to in the review
- Annex 2 Intakes of vitamin E from food and supplements in the UK
- Annex 3 Summary table of selected nutrition related information and existing guidance on intakes

Expert Group on Vitamins and Minerals Secretariat
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VITAMIN E

Chemistry

1. The term vitamin E is used as a generic designation for a group of eight structurally related compounds synthesized by plants (Basu and Dickerson 1996). These compounds fall into two classes, tocopherols and tocotrienols, which exhibit the biological antioxidant activity of vitamin E. Their basic structure consists of a hydroxylated ring system (chromanol ring) and an isoprenoid side chain. The isoprenoid side chain is saturated in the tocopherols, whilst it is unsaturated in the tocotrienols. Unlike the tocopherols which exist only as free alcohols, the tocotrienols can occur naturally in their esterified form. Vitamins in both classes are designated by the Greek letters α , β , γ and δ . Biologically the most active antioxidant is D- α -tocopherol (COMA 1991) accounting for 90% of the vitamin E found in human tissues.

Natural Occurrence

2. Vitamin E is synthesized only by plants (Basu and Dickerson, 1996) and is therefore found primarily in plant products, the richest source being plant oils. All higher plants appear to contain α -tocopherol in their leaves and other green parts, while γ -tocopherol is generally present in lower concentrations. Green plants tend to contain more vitamin E than yellow ones, as α -tocopherol is found in the chloroplasts of plant cells, whilst the β -, γ - and δ -tocopherols are usually found outside these organelles. In contrast the tocotrienols are not found in green leaves, but in the bran and germ fraction of certain plants.
3. Animal tissues tend to have low amounts of vitamin E. The highest levels occur in fatty tissues and vary according to the intake of vitamin E.

Occurrence in foods, food supplements and medicines

Food

4. Vitamin E is synthesized only by plants, so plant oils are the main dietary sources of vitamin E (560-1600 mg/kg in soybean oil, 530-1620 mg/kg in corn oil and 50-150 mg/kg in olive oil), with meat (0.5-1.6 mg/kg), poultry (1.6-4.) mg/kg) and dairy products (0.4-10.0 mg/kg) providing only moderate amounts (Basu and Dickerson, 1996). The amount of vitamin E in foods at the point of consumption is difficult to assess as it depends upon the effects of processing, storage and preparation. Due to the fact that vitamin E is fat soluble it is not lost by leaching into processing water and is generally stable (Basu and Dickerson, 1996). However, being an antioxidant in many vegetable oils, it is destroyed under oxidising conditions, such as exposure to air and light, this is accelerated by heat and in the presence of transition metal ions. Such changes are relatively slow.

5. The biological potency of food should take account of the total tocopherol content as well as the α -tocopherol content. It is usual to express vitamin E activity as α -tocopherol equivalents, the biological potency of α -tocopherol is 1, that of γ -tocopherol 0.08; α -tocotrienol is 0.21 and γ -tocotrienol is 0.01 (Basu and Dickerson 1996). The international unit (IU) of vitamin E is expressed relative to the synthetic form, racemic *all-rac- α -tocopheryl acetate*, where one IU is equivalent to 1 mg based on fetal resorption after oral administration to rats. On this basis, one IU is provided by 0.91 mg of *all-rac- α -tocopherol* and 0.67 mg *RRR- α -tocopherol*.
6. Vitamin E is present in a number of multi-vitamin and/or mineral dietary supplements at levels from 2 to 60 mg (OTC 2001).

Licensed medicinal products for oral use

7. Fifteen medicinal products containing vitamin E (dl- α -tocopherol acetate or succinate) are authorised for sale in supermarkets or other general retail outlets. Another thirteen products are authorised for pharmacy only availability. All are multinutrient products for the prevention and treatment of nutrient deficiency and use by the elderly and those on restricted diets. The doses are up to 20 mg/day.
8. Three high potency products, with daily doses of 100 mg or greater, can be sold without the supervision of a pharmacist for malabsorption disorders such as cystic fibrosis, chronic cholestasis and abetalipoproteinaemia.

Intake and exposure

9. Data from the Dietary and Nutritional Survey of British Adults indicated that average vitamin E intakes from all sources were 11.7 mg for men and 8.6 mg for women (see Annex 2). Supplements of this vitamin were equally important for men and women raising average intakes by 18% and 19% respectively. The main food sources for young people aged 15-18 years were; vegetables, potatoes and savoury snacks (29%), fat spreads (21%) and cereal and cereal products (16%) (see Annex 2).

Recommended amounts

10. The assessment of a dietary requirement for vitamin E is complicated by a number of factors. There is a clear direct relationship between the dietary intake of polyunsaturated fatty acids (PUFAs) and the requirement for the vitamin and there is an equally clear relationship between the tissue content of PUFAs and the dietary requirement. It is commonly found that foods containing large amounts of PUFAs will also contain large amounts of vitamin E. Thus, individuals who consume PUFA-rich foods will also receive a commensurate amount of vitamin E. The mixed diets of individuals in western societies may vary greatly in their

content of PUFAs. Gregory *et al* (1990) gave values for the 2.5 and 97.5 percentile intakes of PUFA by men of 5.1 and 29 mg/day.

11. Due to the widely differing requirements based solely on the PUFA intake, it has been generally considered more realistic to give ranges of acceptable intake rather than a fixed level. This gives a median intake from the survey by Gregory *et al* (1990) of 9.3 mg α -tocopherol equivalent/day for men and 6.7 mg/day for women. COMA (1991) concluded that daily intakes of at least 4 mg and 3 mg of α -tocopherol equivalents would be adequate for men and women respectively. Potential deficiency however, cannot be ruled out if the lower intakes are maintained over prolonged periods. Intakes of 3.8 – 6.2 mg/day appear to be satisfactory for pregnant and lactating women (Black *et al* 1986).
12. Infants that are breastfed have an average daily intake of 2.7 mg α -tocopherol equivalents. In the UK it has been recommended that the vitamin E content of milk formulas should not be less than 0.3 mg tocopherol equivalents/100 ml of reconstituted feed and not less than 0.4 mg/g PUFA (COMA 1991). The Infant Formula and Follow-on Formula Regulations (1995) specify a minimum vitamin E content for both types of product of 0.5 mg α -tocopherol equivalents per gram of polyunsaturated fatty acids expressed as linoleic acid but in no case less than 0.5 mg per 100 available kcal.
13. Other factors known to affect the requirement for vitamin E in the diet include the content of selenium. This trace element is thought to function as an integral part of glutathione peroxidase, an enzyme that reduces toxic lipid peroxides to hydroxy acids (Combs and Combs 1984). Selenium and vitamin E are complementary in terms of their ability to scavenge free radicals. Dietary selenium can thus compensate for vitamin E requirements.

Analysis of tissue levels

14. Measurement of serum plasma concentration of α -tocopherol provides the simplest and most direct evidence of vitamin E deficiency. Values of 5 - 20 μ g/ml for adults and children of twelve years or older and values of 3 - 15 μ g/ml for children under twelve years are acceptable (Basu and Dickerson 1996). Assessment of vitamin E status by measurement of plasma tocopherol concentration is complicated by the fact that increased concentrations of serum lipids appear to cause tocopherol to partition out of cellular membranes into the circulation, thereby increasing blood levels. For this reason, it is more normal to express tocopherol levels in relation to circulating lipids (COMA 1991). If a single lipid is used it is probably most conveniently expressed as the serum tocopherol:cholesterol ratio (COMA 1991). If expressed in relation to serum lipid concentration, then acceptable values are 0.08 mg/g and 0.6 mg/g respectively (Basu and Dickerson 1996). Since the enteric absorption of vitamin E is dependent on the adequate absorption of lipids, individuals with lipid malabsorption syndromes (e.g. cystic fibrosis, biliary atresia, premature infants) typically have low vitamin E status (Combs 1992). At a plasma tocopherol concentration below 0.5 mg/dl (11.6 μ mol/L) or a

tocopherol:cholesterol ratio of about 2.25 $\mu\text{mol}/\text{mmol}$, erythrocytes tend to haemolyse after exposure to oxidising agents which indicates a biochemical deficiency but is not indicative of clinical deficiency (COMA 1991). α -Tocopherol may also be measured in erythrocytes, lymphocytes, platelets, lipoproteins, adipose tissue and buccal mucosal cells or γ -tocopherol may be measured (Morrissey and Sheehy 1999).

15. Another widely used indicator of vitamin E status is the extent of haemolysis of red blood cells in the presence of hydrogen peroxide. A high degree of haemolysis accompanies vitamin E deficiency (i.e. < 20%), however, high peroxidation is not specific for vitamin E deficiency. In addition there are two functional tests which are thought to be of use in assessing vitamin E status in humans. One is an *in vivo* test, in which pentane is measured as a product of PUFA peroxidation in the body. The second is an *in vitro* test in which peroxidation of PUFA of erythrocytes exposed to hydrogen peroxide is measured by determining generated malondialdehyde (COMA 1991, Basu and Dickerson 1996).

Function

16. It has been debated whether vitamin E functions solely as a lipid antioxidant, or whether it may also be required for the function of some other critical, but unknown metabolic factor. Information currently available indicates that all its nutritional effects are consistent with its role as a biological antioxidant. In this regard, vitamin E is thought to have basic functional importance in the maintenance of membrane integrity in virtually all cells of the body. The potent antioxidant properties of vitamin E were first demonstrated by Olcott and Matthill in 1931. It was later proposed that the major function of the vitamin was the protection of PUFAs from oxidation *in vivo* to hydroperoxides. Other oxidation reactions prevented are the conversion of free or protein-bound sulphhydryls to disulphides. However, it was not until more recent years that the precise function of vitamin E was elucidated and its central role in protection against free-radical induced cellular damage was recognised (Chow 1985, Basu and Dickerson 1996).
17. Potentially damaging free radicals are produced in cells under normal conditions either by homolytic cleavage of a covalent bond, or by a univalent oxidation or reduction. The PUFAs of biological membranes are particularly susceptible to attack by free radicals due to their 1,4-pentadiene systems, from which a hydrogen atom is readily removed. The lipoperoxyl free radicals thus formed can attack adjacent PUFA residues and thereby initiate a chain of free radical reactions, with widespread harmful consequences to membrane structure. Vitamin E breaks the chain of free radical formation by reacting with the free radicals and converting them to a non-harmful form. This action, termed free radical 'scavenging', involves the donation of a hydrogen atom to a fatty acyl free radical (or superoxide radical) to prevent the attack of that species on other PUFAs (Lucy 1972, Chow 1985). As noted previously, in the course of this process, α -tocopherol is converted to an α -tocopherol radical which is more stable than fatty acid or peroxy radicals and does not react with membrane PUFA. The α -tocopherol radical can then react

with another radical to form a non radical product or can be re-converted to α -tocopherol (Bramley *et al* 2000).

18. Glutathione levels were increased in the liver, brain, lungs and blood of rats treated with vitamin E (120 mg/kg diet) for 30 days (Boadi *et al* 1991). The addition of selenium or riboflavin did not increase glutathione levels compared to vitamin E alone.
19. Some non-antioxidant functions have been attributed to α but not β -tocopherol (Azzi and Stocker 2000). These include regulation of protein kinase C, modification of cell growth and proliferation, modification of gene transcription, protein phosphatase activation and modifications to gene expression. The authors state that the best evidence for a non-oxidant role is related to the recognition and transfer of α -tocopherol. In the liver, α -tocopherol transfer protein specifically sorts *RRR*- α -tocopherol from other incoming tocopherols for incorporation into plasma lipoproteins. The relative affinities for the other tocopherols are substantially lower, indicating that the stereospecific nature of the binding cannot be due to the anti-oxidant function of the molecule. Additionally, both α and δ -tocopherol are able to induce hepatic mRNA for the α -tocopherol transfer protein, indicating that since the two tocopherols have differing scavenging properties the induction cannot be antioxidant in nature. A dissimilar protection of tocopherol isomers against membrane hydrolysis by phospholipase A2 has also been observed, suggesting biological actions of compounds with vitamin E activity other than their role as antioxidants. Azzi and Stocker (2000) further note that the specific distribution of tocopherols in the tissues indicates a selective role in maintaining cellular functions. The proportion of different tocopherols varies in different tissues and can change ratio in particular situations such as pregnancy.

Deficiency

20. The clinical manifestations of vitamin E deficiency vary considerably between species. In general, however, the targets are the neuromuscular, vascular and reproductive systems. The various signs of vitamin E deficiency are believed to be manifestations of membrane dysfunction, the result of the oxidative degradation of polyunsaturated membrane phospholipids and/or the disruption of other critical cellular processes (Horwitt 1960). In a wide range of animal species, vitamin E deficiency causes an increase in the tendency for erythrocytes to lyse in a solution of hydrogen peroxide. Of the effects of vitamin E deficiency reported in experimental animals, this is the only feature of deficiency which occurs definitely in man and first suggested the possible role of vitamin E in maintenance of membrane stability.
21. Vitamin E deficiency can result from dietary deficiency or impaired absorption of the vitamin. In addition, there is evidence that several other dietary factors affect the need for the vitamin. There is clear evidence that the requirement for vitamin E increases with the amount of dietary PUFAs. Other factors which increase the requirement are deficiencies of sulphur containing amino acids, deficiencies of

copper, zinc and/or manganese and deficiency of riboflavin (Combs 1992). In contrast, selenium decreases the requirement for vitamin E.).

22. The erythrocytes of newborn and premature infants are sensitive to hydrogen peroxide induced haemolysis (Bell 1987). This susceptibility was responsible for the occurrence of haemolytic anaemia reported in premature infants during the 1960s who were fed infant formula containing little vitamin E but abundant PUFA and iron. Improvement in the composition of infant formula has prevented this condition except in rare cases of prolonged parenteral nutrition with lipid emulsion or cases of fat malabsorption resulting from cholestatic liver disease or cystic fibrosis of the pancreas.
23. In experimental animals, vitamin E deficiency has been shown to result in a variety of conditions affecting the neuromuscular, vascular and reproductive systems, as described above. Some conditions, such as the more general ones of loss of appetite, reduced growth and foetal death, besides responding to vitamin E, also respond to selenium and antioxidants. Others, such as myopathy in striated and smooth muscle, liver necrosis, testicular degeneration and exudative diathesis (a defect in blood vessel permeability) in chicks, respond also to selenium. Conditions responding only to vitamin E include encephalomalacia in chicks, cataract in rats and anaemia and intraventricular haemorrhage in premature human infants.

Overview of reported beneficial effects

24. Free radical damage has been implicated in a number of degenerative processes including carcinogenesis, aging, arthritis, platelet hyperaggregability, ischemia and reperfusion injury, cataracts and lung injury caused by pollutants (reviewed Packer 1991). Thus vitamin E may be involved in an extensive range of protective systems. However, a consensus about the exact daily intake of vitamin E for optimal health protection has not been reached. Some people believe that the scientific evidence is strong enough already, especially for cardiovascular disease, to recommend daily intakes in the order of 87-100 mg α -tocopherol equivalents/day (Morrissey and Sheehy 1999).

Cancer

25. In addition to its role as a free radical scavenger, vitamin E may enhance the body's immune function and inhibit the conversion of nitrites to nitrosamines in the stomach (Packer, 1991).
26. As part of the α -tocopherol, β -carotene cancer prevention (ATBC) study more than 29,000 Finnish male smokers, aged 50 – 69 years, were randomly assigned to receive 50 mg α -tocopherol, 20 mg β -carotene, both, or placebo daily for up to eight years. At the end of the study, 246 new cases of prostate cancer and 62 deaths

from the disease had occurred. Incidence was 32% lower and mortality was 41% lower in men taking α -tocopherol, either with or without β -carotene, than in those not taking the vitamin (Heinonen *et al* 1998). Vitamin E also had a small protective effect on the incidence of colorectal cancer, but this was not statistically significant (Albanes *et al* 2000). Higher intakes of vitamin E have been associated with lower risks of various cancers, however there is not enough evidence to conclude that vitamin E protects against the development of various cancers (COMA 1998). Supplementation with 100 mg α -tocopherol acetate twice a day had no effect on oxidative DNA damage in smokers (Priemé *et al* 1997). In an observational study of US health professionals, supplemental vitamin E intake was not generally associated with prostate cancer risk, but there was some evidence of an inverse association with the risk of metastatic and fatal prostate cancer, in current smokers and recent quitters (Chan *et al* 1999).

27. Concomitant vitamin E treatment did not reduce the incidence of colorectal cancer induced by dimethylhydrazine treatment of rats (Hall *et al* 1995). However the ratio of adenomas to carcinomas was altered suggesting that vitamin E treatment retarded the progression from adenoma to carcinoma. Vitamin E has been reported to reduce the incidence of skin tumours induced by 7,12-dimethylbenzanthracene or croton oil (discussed in Kappus and Diplock 1992).

Cardiovascular Disease

28. Vitamin E is thought to have a role in prevention of atherosclerosis, through inhibition of oxidation of low-density lipoprotein. Resistance of LDL to oxidation has been demonstrated in volunteers supplemented with 1600 mg/day vitamin E (Reaven *et al* 1993). Vitamin E is reported to have other beneficial effects on cardiovascular disease, modulating the arachidonate cascade to reduce vasoconstriction and preventing smooth muscle cell proliferation (Bramley *et al* 2000). Some epidemiological studies have shown an association between high dietary intake or high serum concentrations of α -tocopherol and lower rates of ischaemic heart disease. In a review by Stampfer and Rimm (1995) the results of a number of major observational studies were considered, these included the Nurses' Health Study and the Health Professionals Follow up Study. The authors concluded that use of vitamin E supplements for at least 2 years was associated with a reduced risk of coronary heart disease, but that short duration or low doses (<100 IU/day) had no significant effect. The effect of dietary vitamin E was modest and non-significant. In a double-blind, placebo-controlled study (the CHAOS study), patients with proven coronary atherosclerosis were followed. It was concluded that, α -tocopherol treatment substantially reduced the rate of non-fatal myocardial infarction in these patients, with beneficial effects apparent after one year of treatment (Stephens *et al* 1996). However, Vitamin E was not associated with a decreased risk of myocardial infarction in elderly subjects in the Rotterdam cohort study (Klipstein-Grobusch *et al* 1999). However, the differences between high and low intakes were modest with only a few subjects using supplements. COMA (1994) concluded that the evidence for a protective effect of vitamin E on cardiovascular disease was persuasive but not yet conclusive.

Dietary supplementation with vitamin E after myocardial infarction had no beneficial effects on fatal cardiovascular events (GISSI-Prevenzione Investigators 1999).

29. The effectiveness of vitamin E as an inhibitor of platelet adhesion was tested *in vitro* with plasma from six adult volunteers whose diets were supplemented with 200 IU and 400 IU a day for alternate 2 week periods (Jandak 1989). The results indicated that α tocopherol decreased platelet adhesion possibly through the inhibition of pseudopodium formation. Although, the vitamin inhibited platelet aggregation *in vitro*, this was not repeated *in vivo* at a supplementation level of 1200 IU a day (Steiner 1993).
30. A number of studies have examined vitamin E supplementation and benefits for individuals with diabetes mellitus. The evidence that those with Type I diabetes have an increased susceptibility to plasma LDL oxidation is inconclusive (Astley 1999). A randomised placebo controlled trial of 42 patients with Type I diabetes whose diets were supplemented with 400 IU a day of vitamin E found that the susceptibility to LDL oxidation was reduced in the controls but not in the study population (Astley 1999). A double blind clinical trial of 29 children with Type I diabetes (Jain *et al* 2000) found glutathione to be significantly related to vitamin E level and daily supplementation (100 IU) increased glutathione concentrations, lowered lipid peroxidation and HbA_{1c} concentrations in erythrocytes.
31. Measurements of ethane, as a marker of lipid peroxidation, showed that rats fed ethanol exhaled higher levels than their counterparts (Mufti and Ekelson 1991). Generation of ethane indicates a continuing process of free radical attack on lipids. However, exhalation of ethane in rats co-administered vitamin E (400 mg/kg bw/day for two days) decreased to a level intermediate to that of the treated and control groups.

Other Conditions

32. Vitamin E has been reported to successfully relieve the symptoms of fibrocystic breast disease (Gonzalez 1980). Women with fibrocystic breast disease are thought to be at a two to eight-fold greater risk of development of breast cancer. The mechanism of action of the vitamin is unknown, but it is thought to alter blood levels of various hormones, including the adrenal androgens and the gonadotrophins. Vitamin E has also been reported to relieve menopausal symptoms such as hot flushes, sweats, vertigo, headaches, parasthesia, fatigue, insomnia and “nervousness” (Finkler 1949, McLaren 1949).
33. It has been reported that vitamin E has shown the most consistent and greatest effect on AIDS of all the antioxidants (Liang *et al* 1996, Wang and Watson 1994, Tang *et al* 1997). Additionally, antioxidant nutrients including vitamin E may be effective in slowing the progression of Parkinson’s disease in those not already receiving medication. Those patients receiving 3000 mg vitamin C and 3200 IU vitamin E required medication considerably later than those who did not receive

this treatment (Fahn 1992). Vitamin E has been reported to be beneficial in treating a sub-group of patients with tardive dyskinesia caused by dopamine-blocking neuroleptic drugs (Boomershine *et al* 1999). In a review (Behl 1999) it is suggested that vitamin E may be protective against Alzheimer's Disease through a mechanism in which it inhibits the accumulation of the amyloid beta peptide that has been implicated in the pathogenesis of Alzheimer's.

34. Vitamin E is used to treat scleroderma and may prevent retrolental fibroplasia and intracranial haemorrhage in premature infants (Bell 1987).

Interactions

Vitamin K

35. Although vitamin E alone has no measurable effect on coagulation in normal animals and man, it can exacerbate the vitamin K deficiency caused by dietary deficiency or warfarin therapy (Corrigan 1982). In chicks, treatment with excess vitamin E resulted in increased prothrombin time, which was reversed by vitamin K treatment (March *et al* 1972). In patients taking megadoses of vitamin E (up to 26 times the RDA), the coumarin-induced reduction in vitamin K-dependent clotting factors is enhanced (Corrigan and Marcus 1974, Schrogie 1975, Olson 1984). The same effect has also been demonstrated in laboratory animals (Schrogie 1975). It has not yet been determined if the vitamin E-vitamin K interaction is at the level of absorption or metabolism. In a study by Korsan-Bengsten *et al* (1974), nine post-myocardial infarction patients were treated with 300 mg/day α -tocopherol. After 18, 44 and 64 weeks a highly significant increase in clotting time was apparent. There was no change in factor II, VII or X activity; a decrease in platelet factor III activity was thought to be responsible for the increased clotting time. However, this has not been repeated in other studies with different doses and lengths of treatment (Anonymous 1983). It has been suggested that vitamin E could interfere with the oxidation of vitamin K hydroquinone, depriving the system of the energy needed to drive the carboxylation reaction. In addition, α -tocopherolquinone is a metabolic by-product of vitamin E and a structural analogue of vitamin K hydroquinone, which may competitively inhibit the carboxylation reaction which activates vitamin K dependent clotting factors (March *et al* 1976, Rao and Mason 1975, Bettger and Olson 1982). The effect of vitamin E on coagulation is considered further in the toxicity section of this report.

Ethanol

36. Diet containing 500 mg dl- α -tocopheryl acetate/kg accentuated the fatty changes produced in rats given 20% ethanol in drinking water (Levander *et al* 1973). Conversely, the onset of alcoholic cirrhosis was delayed in animals fed diets deficient in vitamin E.

Bioavailability

37. Vitamin E occurs naturally as the free phenol, whereas in oral supplements esterified forms such as acetates, nicotines or succinates are used. The bioavailability of vitamin E esters is similar to that of the free phenol since they are readily hydrolysed in the gut to free phenol (Bramley 2000).

Absorption

38. α -Tocopherol is absorbed unchanged from the small intestine by non-saturable, passive diffusion. Tocotrienol esters are first hydrolysed by pancreatic esterase (Bjorneboe *et al* 1990). Thus, pancreatic juice and bile are essential for the absorption of vitamin E (Basu and Dickerson 1996). Absorption appears to occur mostly in the upper and middle thirds of the small intestine. The absorption of vitamin E shows biphasic kinetics, which reflect the initial uptake of the vitamin by existing chylomicrons, followed by a time lag, due to the need to assemble new chylomicrons. Absorbed vitamin E, like other hydrophobic substances, enters the lymphatic circulation (Devron 1990).
39. The absorption efficiency of tocopherol and its esters is generally considered to be variable. In human studies over 24 hours, absorption of α -tocopherol and its acetate ester was in the region of 21 - 86% (Gallo-Torres 1980). Limited sample numbers and the variety of experimental approaches taken by different investigators make the interpretation of the results of human studies somewhat complicated (Basu and Dickerson 1996). Moreover, determination of absorption of vitamin E under experimental conditions may give little real indication about the efficiency of absorption of dietary vitamin E.
40. There was no difference between the bioavailabilities of *RRR*- α -tocopheryl acetate and *all rac*- α -tocopheryl acetate given when given in the form of soft gel capsules to human volunteers (Chopra and Bhagavan 1999). The bioavailability of both free and esterified α -tocopherols was not affected by the presence of mixed tocopherols but the bioavailability of γ -tocopherol was reduced by the presence of either free or esterified α -tocopherol.
41. In rats given a single bolus of α -tocopherol intraduodenally, absorption was reported to be approximately 40% (Bjorneboe *et al* 1990). Whereas when α -tocopherol acetate was given as slow continuous infusion into the duodenum absorption was 65% (Traber *et al* 1986). In rats, the appearance of α -tocopherol in the lymph was negligible in the 2-4h following intraduodenal dosing, peaking 4-15h after feeding (Bjorneboe *et al*, 1986). Intestinal absorption via the lymphatic system was 15.4%.

Distribution and metabolism

42. Unlike other fat soluble vitamins such as A and D, vitamin E does not appear to have a specific carrier protein in the plasma. Instead, it is rapidly transferred from

chylomicrons to plasma lipoproteins, to which it binds non-specifically. The vitamin is taken up by the liver and released in low density lipoprotein (LDL) (Traber *et al* 1988, Combs 1992). Most absorbed tocopherols are transported unchanged to the tissues. In non-adipose cells, vitamin E is localised almost exclusively in their membranes. Kinetic studies indicate that such tissues have two pools of the vitamin: a 'labile', rapidly turning over, pool; and a 'fixed', slowly turning over, pool. The labile pools predominate in such tissues as plasma and liver, as the tocopherol contents of those tissues are depleted rapidly under conditions of vitamin E deprivation. In contrast, the adipose vitamin E resides predominately in the bulk lipid phase, which appears to be a fixed pool of the vitamin. Thus, it is only slowly mobilised from this tissue with only long-term physiological significance. Due to the very slow turnover of such 'fixed' pools, the amounts of vitamin E in adipose tissue can be nearly normal in animals showing clinical signs of vitamin E deficiency (Bjorneboe *et al* 1990, Devron *et al* 1991, Combs 1992, Basu and Dickerson 1996).

43. Tissue tocopherol contents tend to be related exponentially to vitamin E intake and unlike most other vitamins, they show no deposition or saturation thresholds. Thus, tissues vary considerably in tocopherol contents (Table 1) and are clearly not related to their lipid content. The proportion of different tocopherols varies in different tissues (discussed in Azzi and Stocker 2000). For example, in the hairless mouse, brain contains virtually all α -tocopherol whereas skin contains nearly 15% tocotrienols. The ratio may also change; the ratio of plasma α - and γ -tocopherol changes during pregnancy and is different from that in non pregnant women (cited in Azzi and Stocker 2000).
44. The antioxidant function of the vitamin results in its oxidation to α -tocopherylquinone, through a semi-stable intermediate, α -tocopheroxyl radical. The first stage in this reaction is reversible, whereas the conversion of the intermediate to the quinone is not. The quinone molecule, which has no vitamin E activity, can be changed by another reversible reaction to the hydroquinone. This can be conjugated with glucuronic acid and the product secreted in the bile or excreted in the faeces (Basu and Dickerson 1996).
45. It is suggested that, because the reaction that produces the tocopheroxyl radical is reversible, there is a possibility that vitamin E may be recycled and that a significant proportion of the vitamin may be regenerated *in vivo* in this way. Two mechanisms have been proposed for this recycling, one with ascorbic acid and the other with thiol as the reducing agent (Combs 1992). There is little evidence to support the suggestion that ascorbic acid is involved in this role. However, in support of the second mechanism, it has been shown that the reduced form of glutathione (GSH) can reduce the membrane-bound tocopheroxyl radical in the presence of tocopheroxyl reductase activity, found in the endoplasmic reticulum and mitochondria (COMA 1991, Basu and Dickerson 1996).

Table 1. Concentrations of α -tocopherol in human tissues and body fluids

Tissue	α -tocopherol	
	$\mu\text{g/g}$ tissue	$\mu\text{g/g}$ lipid
Plasma	9.5	1.4
Erythrocytes	2.3	0.5
Platelets	30	1.3
Adipose tissue	150	0.2
Kidney	7	0.3
Liver	13	0.3
Muscle	19	0.4
Heart	20	0.7
Uterus	9	0.7
Ovary	11	0.6
Testis	40	1.0
Adrenal	132	0.7
Hypophysis	40	1.2

Source: Machlin 1984

Excretion

46. At normal levels of intake, the vitamin is conjugated with glucuronic acid and excreted, via the bile, in the faeces. The level excreted is considerable ranging from 30-70% (Bramley *et al* 2000). Less than 1% of vitamin E is excreted in the urine under these circumstances. The urinary metabolites that have been identified are α -tocopheronic acid and α -tocopheronolactone. These are side-chain oxidation products of tocopherol and may be present as conjugates of glucuronic acid (Chow 1985, Meyers *et al* 1996). Some vitamin E may be eliminated via the skin.

Toxicity

47. Vitamin E is one of the least toxic of the vitamins. Animals and humans appear to be able to tolerate high levels of the vitamin (i.e. at least two orders of magnitude above nutritional requirements, e.g. 1000 – 2000 IU/kg diet) without untoward effects (Combs 1992). At very high doses, however, vitamin E can produce signs indicative of antagonism with the function of the other fat-soluble vitamins (Bendich and Machlin 1988). Thus, animals with hypervitaminosis E have been found to show impaired bone mineralisation, reduced hepatic storage of vitamin A and coagulopathies. In each case, these signs could be corrected with increased dietary supplements of the appropriate vitamin (i.e. vitamins D, A and K, respectively).

*Human toxicity**Case Reports*

48. Isolated reports of negative effects in humans consuming up to 3200 IU of vitamin E per day include headache, fatigue, nausea, double vision, muscle weakness, mild creatinuria (84 and 71 mg/24 hours at day 7 of treatment and 98 and 121 mg/24 hours after 14 days treatment in one study and 151 and 143 mg/24 hours) and gastrointestinal distress (cited Myers *et al* 1996). In a review by Roberts, (1981) other sporadic symptoms noted, include thrombophlebitis, hypertension, thyroid dysfunction, hypercholesterolaemia, hypertriglyceridaemia, alterations in hormone status, vaginal bleeding, stomatitis, urticaria, retarded wound healing, vertigo diarrhoea, intestinal cramps, gynaecomastia and breast symptoms. It has also been suggested that vitamin E could interfere with steroid hormone production by inducing cytochrome P450 production (Briggs and Briggs 1974).

Other Studies

49. Eight healthy young men volunteered to take part in a double blind study of 800 IU vitamin E as D- α -tocopheryl acetate or placebo (Briggs, 1974). After three weeks, 2 volunteers asked to withdraw from the trial due to severe fatigue and weakness. When the code was broken, both were found to be in the treatment group. Both subjects showed elevated serum creatine kinase levels at days 7 and 14, accompanied by creatinuria. These parameters returned to normal after treatment was stopped. Biochemical measurements were normal in the other subjects in the study. This was published in response to a letter by Cohen (1973) describing anecdotal reports of fatigue following vitamin E supplementation. In contrast, Ayres and Mihan (1974) also responded, but reported no such symptoms in more than 125 patients that they had treated with vitamin E.
50. In a group of 28 adults voluntarily ingesting 100 to 800 IU/day of α -tocopherol for an average of three years, no apparent toxic effects were noted (Farrell and Bieri 1975). A slight increase in serum lipid levels was observed, the increase being related to the α -tocopherol level.
51. As noted previously, there is evidence for an interaction between vitamin E and K. This may be due to inhibition of vitamin K-dependent carboxylation with the subsequent deleterious effect on clotting factors. Patients given large doses of vitamin E (i.e. 1200 IU/day) showed prolonged blood clotting times due to hypoprothrombinaemia (Horwitt 1980, Combs 1992). This effect may only be of concern to patients on anti-coagulant therapy. The metabolic basis of this effect is not clear, it is thought to involve tocopherylquinone which, bearing structural similarities to vitamin K, may act as inhibitor of vitamin K metabolism.
52. Some trials investigating the effect of vitamin E on intra-cranial haemorrhage in premature infants have reported an increased incidence of intraventricular and retinal haemorrhage in infants given *i.v.* tocopherol, this may result from the

known effects of vitamin E on coagulation (Bell 1987). An *i.v.* preparation of [OIS1] α -tocopheryl acetate administered to premature infants was associated with a syndrome of ascites, liver and kidney failure which led to the deaths of 38 infants and the serious illness of many others.

53. Roberts (1978) reported that in the course of 10 years he had treated 46 patients with thrombophlebitis who had been taking vitamin E supplements (the majority taking a dose of 400-800 IU, for at least 1 year). Symptoms generally disappeared following the cessation of vitamin E treatment and with conventional treatment for thrombophlebitis. However, the condition persisted for some months in some individuals and exacerbation of the condition was observed in two patients challenged with vitamin E, 2 months later.

Genotoxicity

54. Thirty one healthy volunteers were randomised to receive 15, 60, or 200 mg/day vitamin E or a placebo for 4 weeks (Hu *et al* 1996). Levels of lipid and water antioxidants and DNA repair activities in peripheral mononuclear leukocytes were assessed at baseline and on days 17 and 31 (dosing started on day 3). Plasma tocopherol was significantly increased in the top two dose groups and on day 31 of the study plasma glutathione levels were elevated. No adverse effects were reported and no dropouts occurred.
55. Healthy adult volunteers were supplemented with 1000 mg/day vitamin E for 6 weeks (Brennan *et al* 2000). Hydrogen peroxide-induced DNA damage in control and treated peripheral blood lymphocytes was assessed by ELISA during a post-treatment washout period. Vitamin E supplementation resulted in a significant decrease in DNA damage compared to the controls, the effect gradually reducing during the washout period. The treatment had no effect on levels of endogenous damage. The activities of the antioxidant enzymes superoxide dismutase and glutathione peroxidase were suppressed during the supplementation period. However, in a double blind placebo controlled trial conducted by Huang and colleagues (2000) supplementation of non smoking volunteers with 400 IU vitamin E, vitamin C or both vitamins did not affect oxidative DNA damages as measured by urinary excretion of 8-hydroxy-2'-dehydroxyguanosine. Fruit and vegetable intake, however, and serum ascorbic acid levels were inversely associated with urinary 8-hydroxy-2'-dehydroxyguanosine.

Human Supplementation Studies

56. A variety of studies considering the effects are considered below. This is not exhaustive but has tried to include studies using higher level of vitamin E or long dose periods, or where side effects or biological parameters were specifically investigated.
57. The effect of vitamin E on serum lipids was investigated in a double blind placebo controlled study (Stampfer *et al* 1983). Groups of fifteen adults aged 30-60

received 800 IU/day or placebo for 16 weeks. High density lipoprotein cholesterol, total cholesterol and triglyceride levels were measured at baseline, 8 and 16 weeks. A marginal decrease in low-density lipoprotein cholesterol occurred in the treatment group at week 16 but no other changes in serum lipids were observed. No adverse effects were noted.

58. Thirty elderly subjects were included in a double-blind placebo-controlled study with a crossover design (Paolisso *et al* 1995). The subjects received 900 mg/day or placebo for 4 months; one month run-in and wash out periods were also used. Vitamin E administration was associated with a lowering in fasting plasma insulin levels, plasma triglyceride concentrations, and the ratio of plasma LDL to HDL. Vitamin E treatment was also associated with an increase in non-oxidative glucose metabolism during a euglycaemic glucose clamp. It was stated that none of the patients dropped out of the study during vitamin E administration, or experienced side effects that were likely to be related to vitamin E treatment (it is unclear how the lack of side effects was ascertained). Liver and renal functions were the same post-treatment as during the run-in and placebo periods.
59. In the alpha-tocopherol, beta-carotene (ATBC) study, a total of 29,133 Finnish male smokers were randomly assigned to one of four treatment groups: 50 mg α -tocopherol/day, 20 mg/day β -carotene, both α -tocopherol and β -carotene, or placebo (Albanes *et al* 1994). Follow up continued for five to eight years. Lung cancer incidence was not affected by α -tocopherol treatment, but the incidence of prostate cancer was reduced. α -Tocopherol had no effect on total mortality but was associated with an increase in mortality from haemorrhagic stroke, 7.8 deaths per 10,000 person years in the α -tocopherol group versus 5.2 deaths per 10,000 person years in the groups who received no α -tocopherol. It should be noted that this study was conducted in a high-risk group for haemorrhagic stroke, i.e. long term heavy smokers (WHO 1995). Deaths from ischaemic stroke and ischaemic heart disease were reduced in the α -tocopherol group. Minor side effects were not reported. The effects of α -tocopherol on stroke was analysed with regard to baseline, systolic blood pressure, serum total and HDL cholesterol levels, histories of diabetes and heart disease, cigarette smoking, alcohol consumption and physical activity and in connection with high risk for specific stroke (Leppälä *et al* 2000a). Of all the risk factors, only systolic blood pressure modified the effect of vitamin E. Vitamin E increased the risk of subarachnoid haemorrhage and decreased the risk of cerebral infarction in hypertensive men but had no effect on normotensive men. Vitamin E supplementation, however, decreased the risk of cerebral infarction without elevating the risk of subarachnoid haemorrhage among hypertensive men with concurrent diabetes. It has been suggested (Leppälä *et al* 2000b) that the effect of vitamin E on stroke is due to the anti-platelet and anti-coagulant effect of vitamin E; the authors note that vitamin E and aspirin are more effective than aspirin alone in preventing ischaemic stroke.
60. The incidence of primary non-fatal myocardial infarction and fatal coronary heart disease was investigated in the same study group (Virtamo *et al* 1998). In subjects taking α -tocopherol, a small decrease (4%) in primary major coronary events was

observed, as was a 9% decrease in fatal coronary heart disease but the incidence of non-fatal myocardial infarction was not affected by treatment.

61. In the Cambridge Heart Antioxidant Study (CHAOS) 2002 patients with atherosclerosis were entered into a double-blind placebo-controlled study and followed up for a median of 510 days (range 3-981) (Stephens *et al* 1996). The treatment group received a dose of 800 IU vitamin E/day for the first 546 patients and 400 IU/day for the remainder. Treatment significantly reduced the risk of cardiovascular death and non-fatal myocardial infarction (a composite endpoint) (41 events vs. 64, RR 0.53, 95% CI 0.34-0.83, p=0.005). However, this decrease was due to the decrease in MI since, when separated out, there was a non-significant excess of cardiovascular deaths in the treatment group (27 events vs. 23, RR 1.18, 95% CI 0.62-2.27, p=0.61). The increased risk appeared to be due to an excess of early events (before 200 days). The authors speculate that the finding might be due to chance or might reflect a difference in biological events leading to death and those leading to non-fatal MI and note that the results of larger studies would be necessary to clarify this. The study was not designed to look at any dose response effects and there was no randomisation of the subjects within the different treatment groups.
62. In the Gissi-Prevenzione study, 11, 324 patients who had survived recent myocardial infarction were randomly assigned supplements of n-3 PUFA (1g daily), 300 mg vitamin E (as synthetic α -tocopherol), both or neither for 3.5 years (Gissi-Prevenzione investigators, 1999). The primary combined efficacy endpoint was death, non-fatal myocardial infarction and stroke. Smokers and ex-smokers were evenly distributed through the groups. Vitamin E treatment had no effect on the combined or separate endpoints.
63. In the Heart Outcomes Prevention Evaluation (HOPE) study (Yusuf *et al.*, 2000), a total of 9,541 subjects (6996 men and 2545 women) aged 55 or over at high risk for cardiovascular events were enrolled in a trial with a 2x2 factorial design. The participants received either 400 IU vitamin E (from natural sources) or placebo and either ramipril or matching placebo for a mean of 4.5 years. The primary endpoint was a combination of myocardial infarction and stroke and death from cardiovascular causes. The secondary outcomes included unstable angina, congestive heart failure, revascularisation or amputation, death from any cause, complications of diabetes and cancer. There were no significant differences in the numbers of deaths from cardiovascular causes (RR 1.05; 95%CI 0.9-1.22) between those receiving vitamin E or placebo or in any of the secondary outcomes.
64. In a controlled open 2 x 2 factorial trial, 4495 people were randomised to receive low dose aspirin (100 mg/day) or no aspirin, and vitamin E (300 mg/day as synthetic α -tocopherol) or no vitamin E to investigate the prevention of cardiovascular events in people with one or more major cardiovascular risk factors (hypertension, hypercholesterolaemia, diabetes obesity or family history) (Primary Prevention Project, 2001). The mean follow up period was 3.6 years. The main combined endpoint was the cumulative rate of cardiovascular death, non-fatal

myocardial infarction and non-fatal stroke. Predefined analyses included cardiovascular deaths, total deaths, and total cardiovascular events. Smokers and ex-smokers were evenly distributed through the groups. Vitamin E had no effect on any pre-specified endpoint.

65. In a randomised placebo-controlled trial of antioxidant vitamin supplementation, 20,536 adults with coronary heart disease, other occlusive vascular disease or diabetes were given a daily supplement of 600 mg α -tocopherol, 250 mg vitamin C and 20 mg β -carotene or placebo for five years (Heart Protection Study Collaborative Group, 2002). No difference in all cause mortality was revealed. Compliance was 83% on average in each treatment group, adverse experiences were sought at each follow up visit (every 4 months for the first year and every 6 months thereafter) and no significant side effects were reported. In particular, there was no significant difference on the number having a haemorrhagic stroke. Current and ex-smokers were evenly distributed between groups.
66. Oral α -tocopherol (400 mg/day) was given to 10 healthy volunteers for 4 weeks (Ernst and Matrai 1985). Blood and plasma viscosity, red cell deformability, red cell aggregation, colloid osmotic pressure of plasma, blood count, total lipids and α -tocopherol levels were measured at baseline, 2 and 4 weeks. The results showed a significant increase in red cell deformability and α -tocopherol levels.
67. Twenty-eight subjects with insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) were randomly assigned to receive 1200 IU (1632 mg) *RRR*- α -tocopheryl acetate for 8 weeks (Fuller *et al* 1996). Compared to the placebo, the treatment group had a significant reduction in red cell oxidizability (measured by conjugated diene formation and lipid peroxidation) and the lag phase of the assays was also increased. No significant changes in glycated haemoglobin or in glycated plasma occurred. No adverse effects were reported.
68. Groups of eight healthy male subjects per dose were randomised to receive placebo, 60, 200, 400, 800 or 1200 IU vitamin E/day (Jialal *et al* 1995) for 8 weeks. The aim of the study was to investigate the dose response relationship of vitamin E and LDL oxidation. A decreased susceptibility of LDL to oxidation occurred at doses greater than 400 IU/day. It was stated that no side effects were experienced as determined by clinical examination or routine laboratory analysis. There were no deleterious effects on the plasma lipid and lipoprotein profile of the volunteers. No drop-outs from the study occurred.
69. Forty eight patients with stable angina completed a double-blind crossover study of two 6 month periods of treatment with 1600 IU/d α -tocopherol or placebo (Gillilan *et al* 1977). No statistically significant differences were apparent in a number of cardiac parameters. The subjects were questioned regarding possible side effects and underwent periodic urinalysis, blood chemistry analysis and complete blood count as well as measures of prothrombin time, a chest X-ray and ECG. No deleterious side effects were observed. There was a slightly increased incidence of

gastrointestinal disturbance during the placebo phase. There was no exacerbation of hypertension, congestive heart failure or skeletal muscle complaints could be attributed to treatment. No differences were found in the various clinical chemistry studies.

70. In the first part of a double-blind placebo-controlled study, two groups of twenty patients received 3200 IU vitamin E or placebo for 9 weeks (Anderson 1974). In the second part of the trial, patients who were already taking vitamin E supplements of 400 to 2400 IU vitamin E received the same (or larger) dose of vitamin E (n=8) or placebo (n=7). The actual dose regime is unclear for this part of the study. The authors stated that overall, the study did not provide statistically convincing evidence that vitamin E was useful in the treatment of angina but a small beneficial effect could not be ruled out. Two patients withdrew from the first part of the trial as a result of heartburn (placebo) and diarrhoea (treatment). One patient (treatment group) withdrew from the second part of the study due to intestinal cramps. Including the dropouts, the side effects reported were: nausea (1 treatment, 2 placebo), heartburn (2 placebo), diarrhoea (2 treatment), intestinal cramps (1 treatment), and tiredness and itching (1 placebo). The authors considered that the only symptoms attributable to treatment were those of the gastrointestinal tract.
71. Eighty-eight, healthy older subjects (aged > 65y) were entered into a double-blind placebo-controlled study, receiving placebo, 60, 200 or 800 IU (55, 182 or 727 mg) vitamin E/day for four months (Meydani *et al* 1998). The groups contained 17, 19, 18 and 19 subjects respectively. The study was designed to assess the effect of supplementation on general health and measured an extensive range of parameters. It is stated that no side effects were reported. Supplementation had no effect on plasma concentration of other anti-oxidant vitamins and minerals, glutathione peroxidase, superoxide dismutase or total cysteine. There was no significant effect of vitamin E on serum non-specific immunoglobulin concentrations or anti-DNA and anti-thyroglobulin antibodies. The cytotoxic ability of neutrophils against *Candida albicans* was not compromised. Vitamin E had no effect on body weight, plasma total proteins, albumin, glucose, plasma lipids or the lipoprotein profile, total bilirubin, serum liver enzymes, blood count, platelet number, bleeding time, haemoglobin, haematocrit, urinary or serum creatine levels. The authors concluded that supplementation had no detrimental effect on health.
72. Elderly subjects were assessed by questionnaire regarding their health and diet (Hale *et al* 1986). During a 2-year period, information was available on 369 supplement users and 1,861 non-users. There was no difference in the health of the participants except that vitamin E users were more likely to complain of shortness of breath and angina. Blood samples were taken at a clinic and tested for a range of clinical chemistry and haematology parameters. Once the group had been adjusted for age and sex, there were no significant differences with the exception of serum glutamic-oxaloacetic acid (SGOT) which was significantly elevated in men.

73. In a double blind, placebo-controlled crossover study, 36 type 1 diabetic subjects and 9 non-diabetic were given 1,800 IU vitamin E a day as for 4 month and placebo for 4 months to assess the effect of vitamin E on retinal blood flow and creatinine clearance (Bursell *et al* 1999). Vitamin E treatment normalised both baseline creatinine clearance and retinal blood flow in the diabetic patients compared to the non-diabetic controls. One diabetic patient receiving vitamin E was taken out of the study due to the development of low thyroid activity. Other adverse events reported were four of dizziness, one of headache, two of breast pain, one of fatigue and one of diarrhea. These were not considered to be serious and no association with vitamin E treatment was discernable. During the study there was no significant differences between diabetic and non-diabetic subjects in clinical laboratory values, liver function lipids and blood chemistry both within and between groups. It was noted that, for example, serum creatinine in diabetic patients was not affected by treatment. Vitamin E treatment was associated with a non-significant increase in cholesterol levels.
74. Twenty five patients on chronic warfarin therapy were identified and included in a two-phase double blind study (Kim and White 1996). In the first phase, 12 patients were randomised to receive 800 IU vitamin E, 1,200 IU or placebo for 1 month. The international normalised ratio was measured twice every week. Defined increases in the INR were considered to be possible or probable drug effects. Such effects were not observed in the treatment group. In the second, unblinded, phase of the study additional subjects were given 1,200 IU vitamin E or placebo and also followed for 1 month. In the second phase of the study, the 6 patients given vitamin E had stable INR measurements. The precise number of controls in this phase is unclear, but appears to be 4. A number of the original study population identified were subsequently excluded for not meeting the entrance criteria of having an INR in the target therapeutic range. The authors concluded that patients on warfarin could safely be given moderate to large doses of vitamin E, provided INR was tested 1-2 weeks after starting treatment.

Epidemiological Studies

75. As part of the Nurses' Health Study, in which a total of 87, 245 female nurses aged 34-59 were recruited and followed up for up to 8 years, the effect of vitamin E consumption on coronary disease was assessed (Stampfer *et al* 1993). The risk of major coronary disease was reduced in the fifth of the group with the highest vitamin E intake (median of 208 IU/day) compared with the fifth with the lowest intake (median of 2.8 IU/day). Most of the variability in intake and reduction in risk was attributed to the use of supplements. Little benefit was seen in women taking supplements for short periods of time, whereas those taking supplements for at least 2 years had a relative risk of major coronary disease of 0.59 after adjustment for age, smoking, risk factors for coronary disease and the use of other anti-oxidants.
76. In the Health Professional Follow up Study, 43, 738 men were recruited and followed up for up to 8 years (Ascherio *et al* 1999). Vitamin E intake was assessed

by food frequency questionnaire which included questions on supplement use. None of the subjects had cardiovascular disease or diabetes. After adjustment, the relative risks for ischaemic and total stroke were not affected by vitamin E intake. The association of vitamin E with haemorrhagic stroke was also non-significant but the confidence intervals were wide. The authors concluded that vitamin E supplements did not affect stroke risk but that modest effects could not be ruled out.

Adverse Drug Reactions

77. Most of the reactions reported to the UK Medicines Control Agency relate to multi-constituent products, and may not therefore be attributable to vitamin E. A small number of reactions have been reported for single constituent products, but none showed any trends suggestive of an association with vitamin E treatment.

Vulnerable Groups

Genetic Variants

78. Familial isolated vitamin E deficiency is an uncommon autosomal recessive neurodegenerative disease, whose clinical presentation is similar to that of Friedrich ataxia (discussed in Azzi and Stocker 2000). It is caused by mutations in the gene for α -tocopherol transfer protein. Therapeutic and prophylactic supplementation with vitamin E can prevent the onset of the disease before irreversible damage develops.

Animal toxicity

Acute Toxicity

79. Newborn rabbits, fed intravenously, received 100 mg daily of either α -tocopherol or α -tocopherol acetate by intravenous injection for 6 or 7 days. Animals treated with α -tocopherol acetate had microscopic evidence of mild bile stasis and had elevated serum bilirubin. Minimal lipidosis or fatty change in the liver was observed in both treatment groups. Lipidosis in the spleen was moderate in pups treated with α -tocopherol and minimal in the α -tocopherol acetate treated pups. Lipidosis also occurred in the adrenal gland, primarily in the α -tocopherol acetate group (Rivera *et al* 1990).
80. The acute oral LD₅₀ for d- α -tocopherol poly (ethylene glycol) 1000 succinate (TPGS) in young adult rats is >7000mg/kg bw (Krasavage and Terhaar 1977). The TPGS was given by gavage. After a 24 hour period of lassitude and diarrhoea, no gross changes in behaviour, coat or stool were observed. In the 2.5 weeks following treatment, no adverse effects on body weight were apparent in the surviving (3 gavage deaths occurred) treated animals.

Sub-chronic Toxicity

81. A thirteen week study was conducted (Abdo *et al* 1986) in which α -tocopherol acetate was administered in corn oil by oral gavage to groups of ten male and female rats at doses of 0, 125, 500 or 2000 mg/kg bw/day. Vitamin E dosing had no effect on body weight or food consumption. The liver to bodyweight ratio of females at 500 and 2000 mg/kg was significantly increased. Treatment related deaths occurred only in male rats at the 2000 mg/kg dose level. Seven out of ten animals in this dose group died or were killed in a moribund state during weeks 9–11 of the study. In males, high levels of vitamin E (2,000 mg/kg) caused prolongation of both prothrombin and activated partial thromboplastin times, reticulocytosis and a decrease in haemocrit values and haemoglobin concentrations. Activated partial thromboplastin times were also increased in females at the highest dose level and in males in the 500 mg/kg dose group. High levels (2,000 mg/kg) of vitamin E caused haemorrhagic diathesis in both males and females and increased medullary erythropoiesis in the spleen of one male.
82. Fifteen rats/sex/group were fed diet containing 0, 0.002, 0.02 or 2% d- α -tocopherol poly (ethylene glycol) 1000 succinate (TPGS) for 90 days (Krasavage and Terhaar 1977). The quantities of TPGS ingested were 0.32-0.48, 31.5-45.6 and 316.8-443.1 mg/rat respectively. The treatment had no effects on body weight gain, behaviour or appearance. Haematology and clinical chemistry parameters were measured in control and high dose animals; but no differences were apparent. No dose related changes in organ weights were observed. Microscopic pathology was conducted on the control and high dose animals only and the results were considered to be unremarkable.
83. It has been reported that rats and mice can tolerate doses of 4 and 50 g/kg bw/day vitamin E for 2 months respectively (Demole *et al* cited by JECFA¹).
84. Rats fed doses of 100 mg/kg bw/day α -tocopherol for 19 weeks showed an increase in phosphorus metabolism as measured by increases in the concentration of phosphorus in a range of tissues. The increases ranged from 14 to 161 % depending on the age of the rat and the tissue measured. No significant effects were observed in rats treated with 10 mg/kg bw/day (Weissburger and Harris 1943). Vitamin E deficiency also stimulated phosphorus metabolism though to a lesser extent. No other effects related to treatment were reported.
85. Rats treated with weekly oral doses of 150 mg of a vitamin E concentrate for 20-27 weeks had fatty changes in the liver, and intimal sclerosis of the aorta with over-development of the tissue at the base of the aortic valve and in the medial coat of the aorta (Marx *et al* 1947). Cholesterol levels in the blood, liver and adrenal glands were unaffected by treatment. The authors note that the vegetable oil concentrate was only 34% vitamin E and other components could have been responsible for the observations.

¹ The original paper is in German and has not been translated for this review.

86. Rats given high doses of α -tocopherol had elevated liver cholesterol levels and altered tissue fatty acids (Alfin-Slater *et al* 1971 cited by JECFA²).
87. Diet containing 500 mg dl- α -tocopheryl acetate/kg had little effect on liver triglycerides, but accentuated the fatty changes produced by ethanol in weanling F344 rats given 20% ethanol in drinking water for 35-39 days (Levander *et al* 1973).
88. Weanling rats were fed a control diet containing 35 mg/kg d- α -tocopherol or a test diet containing 875, 1750, 3500 or 35000 mg/kg for 8 weeks (Dysmsza and Park 1975). The rats in the highest dose group had significantly lower feed and protein efficiencies. Haemoglobin, serum cholesterol and urinary creatine and creatinine were unaffected by treatment but SGPT (serum glutamate pyruvate transaminase) was elevated in the high dose group. It was stated that diet containing 875 and 1750 mg/kg d- α -tocopherol was not associated with adverse effects. This represents a dose of approximately 87.5 and 175 mg/kg bw/day (WHO 1987).
89. Growth rates were reduced in chicks fed 2200 IU dl- α -tocopheryl acetate/kg diet for 3-8 weeks (March *et al* 1973). Growth rate was unaffected at doses of 1000 IU/day but depressed at doses of 2200 IU. Thyroid hypertrophy in response to a thiouracil challenge was reduced at the 220 IU/kg level as were thyroidal uptake and release of ¹³¹I suggesting that the hyperplasia necessary to maintain thyroxine production was reduced. The respiration rate of mitochondria from skeletal muscle isolated from chicks fed 2200 IU/kg diet was only 2/3 that of the control mitochondria. Bone calcification was depressed when excess vitamin E was fed to chicks receiving a calcium or vitamin D deficient diet, suggesting that vitamin D increased the requirement for vitamin D. At a level of 2200 IU dl- α -tocopheryl/kg diet, prolonged prothrombin times, reticulocytosis and reduced haematocrit value was apparent. The prolonged prothrombin time was reversed by injection with vitamin K suggesting an increased requirement for vitamin K in the treated animals.

Long Term Studies

90. Groups of weanling female Wistar rats were fed diets containing 0, 25, 250, 2500, 10,000 or 25,000 IU vitamin E (as dl- α -tocopheryl acetate) per Kg diet for 8 and 16 months (Yang and Desai 1977). Body weight gain was depressed at doses of 10,000 IU or above and relative heart and spleen weights were increased in this dose group at 8 and 16 months respectively. In the same dose groups, there was an increase in plasma alkaline phosphatase and a decrease in the ash content of bone after 16 months. Prothrombin time was reduced at 12 months, but not at 9 or 16 months. Urinary excretion of creatine and creatinine was normal at 11 months. No histological examinations were reported. Dietary vitamin E at levels of 2500 IU/kg

² It has not been possible to obtain the original paper.

was associated with no adverse effects. The dose can be estimated to be approximately 125 mg/kg bw/day (WHO 1987).

91. Groups of 50 CD rats/sex/group were fed vitamin E in the diet at doses calculated to provide 500, 1000 or 2000 mg/kg bw/day for 104 weeks (Wheldon *et al* 1983). A further 10 rats/group were killed following 12 months of treatment. The control diet contained 39 mg/kg vitamin E. From weeks 24 onwards, vitamin K supplementation was used to counter an observed haemostatic failure in the treated animals. Difficulty in staunching blood flow was noted at week 8 and frank haemorrhages were observed from weeks 15, 16 and 18 in the high, intermediate and low dose groups respectively. The haemorrhages occurred in the gut, urinary tract, orbit and meninges and from minor trauma of the claws or vibrissal pits. Females were unaffected. Growth rate and survival were unaffected by treatment (however it was noted that causes of death in the treated rats prior to supplementation were related to haemorrhages, whereas in the controls there were other causes). At week 8 only, haematocrit, haemoglobin concentration and erythrocyte count were slightly but significantly decreased in the high dose animals in both males and females so this was not considered to be related to the haemorrhages. There was a trend towards fewer mammary tumours in the females (the same effect was suggested in males but was not significant). Otherwise the tumour profile was not altered by treatment. In the high dose rats, serum alkaline phosphatase was significantly elevated, but this was not consistent and did not occur in other dose groups. A dose-related increase in alanine amino-transferase occurred in weeks 4-26 in the treated males, but losing dose-relation and statistical significance thereafter. Aspartate amino-transferase levels were unaffected as were other clinical chemistry parameters. Because of the changes in serum liver enzyme activity, particular attention was paid to the liver at autopsy. Absolute liver weights were increased in the top dose females only at the interim 12 month kill. At 104 weeks no effects on liver weights were found. No dose related liver micro-pathology was observed with the exception of "foamy macrophages" occurring in treated rats (17% of males and 77% of females), though with no dose relation. A NOAEL cannot be identified from this study.

Reproductive Toxicity

92. Fifteen rats/sex/group were fed diet containing 0, 0.002, 0.02 or 2% d- α -tocopherol poly(ethylene glycol) 1000 succinate (TPGS) for 90 days prior to mating (Krasavage and Terhaar 1977) and then maintained on the same treatment regime. The mean gestation period, litter size, sex ratio and mortality of parents and pups were unaffected by treatment. Consumption of treated diet by the parents and offspring for five weeks after weaning did not affect weight gain. There were no differences in clinical chemistry or haematology between the high dose animals and the controls or in organ weights between control and treated parental animals after 225 days of treatment. Microscopic tissue pathology was also unaffected.

93. Sprague Dawley rats were fed diet containing 5 to 500 mg/kg α -tocopheryl acetate (the doses ranging from 22.5 to 2252 mg/kg bw) during pregnancy and lactation (Martin and Hurley 1977). The rats given diet containing 20 mg/kg vitamin E or greater had higher relative liver weights compared to the controls and rats receiving diet with 500 mg/kg had higher levels of plasma lipids and vitamin E. Teratogenic effects were not observed and the litter size and weight and survival rate of the offspring were unaffected by treatment. It was noted that several of the newborns in the 500 mg/kg dose group showed delayed opening of the eyelids but this was not statistically significant.
94. Fifteen rats/sex/group were fed diet containing 0, 0.002, 0.02 or 2% TPGS from day 6-16 of gestation (Krasavage and Terhaar 1977); a positive control group was also included. No dose related abnormalities were apparent.
95. Pregnant ICR mice were given daily doses of 591 IU α -tocopherol by gavage on days 7-11 of pregnancy (Hook *et al* 1974). In a total of 91 offspring from 7 litters from the treated animals, 1 malformation was observed. This was exencephaly, open eye and micrognathia. In a total of 177 offspring from 13 untreated control litters, or 117 offspring from 8 saline treated control litters, no malformations were observed. The authors state that the malformation had not been described in the strain before, but could be induced by known teratogens.
96. Weanling rats were fed a control diet containing 35 mg/kg d- α -tocopherol or a test diet containing 875, 1750, 3500 or 35000 mg/kg for 8 weeks; the rats were then mated (Dysmsza and Park 1975). Fertility and survival of the pups to weaning was unaffected by treatment, but the number of live pups born was reduced in the highest dose group (approximately 3500 mg/kg bw/day (WHO 1987).

Carcinogenicity

97. No data identified.

Genotoxicity

In vitro

98. No data identified.

In Vivo

99. In the sex-linked recessive lethal test in *Drosophila*, α -tocopheryl acetate at a concentration of 500 IU in the medium did not cause a change in the mutation rate in irradiated male insects, but following mating caused a decrease in the mutation rate in subsequent generations (Beckman *et al* 1982).

Anti-mutagenic Effects

100. Vitamin E has been reported to reduce the mutagenic effects of a number of mutagenic chemicals. Vitamin E reduced the mutagenic effect of malonaldehyde and β -propiolactone in the 5 of 7 *Salmonella typhimurium* strains (hisC207, his3076, TA1977, hisD3052 and TA1978) which mutate through a frameshift mechanism (Shamberger *et al* 1979).
101. In cultures of human lymphocytes, vitamin E did not affect the clastogenicity of bleomycin in the absence of S9 activation but increased clastogenicity in the presence of S9 (Gebhart *et al* 1985). Vitamin E reduced the clastogenicity of trenimon in both the presence and absence of S9 activation but did not affect the activity of S9 activated cyclophosphamide. No anti sister-chromatid exchange effect was found nor was cell proliferation affected by vitamin E treatment.
102. Groups of 20 female hairless pigmented mice were treated with lotion vehicle, 5% topical *RRR*- α -tocopherol, topical *RRR*- α -tocopheryl succinate and oral *RRR*- α -tocopheryl acetate (Burke *et al*, 2000). Within each group, 15/20 mice were also exposed to UV-B radiation three times a week. Mice treated with both oral or topical vitamin E showed no signs of toxicity and had significantly less UV induced acute and chronic skin damage as indicated by reduced inflammation and pigmentation and by later onset of skin cancer.

Regulatory considerations

103. The Infant Formula and Follow-on Formula Regulations (1995) specify a minimum vitamin E content for both types of product of 0.5mg α -tocopherol equivalents per gram of polyunsaturated fatty acids expressed as linoleic acid but in no case less than 0.5 mg per 100 available kcal. The Foods Intended for use in Energy Restricted Diets for Weight Reduction Regulations (1997) recommend that whole diet products should provide 10 mg vitamin E and meal replacement products 3 mg. The Recommended Daily Allowance (for food labelling purposes) for vitamin E is 10 mg.

Existing recommendations on maximum intake levels

104. COMA (1991) concluded that few adverse effects had been reported from doses of vitamin E up to 3200mg/d, and none were observed consistently. JECFA established an ADI of 0.15-2 mg/kg bw for α -tocopherol and mixed tocopherol concentrate (JECFA).

Existing recommendations on maximum supplementation levels

105. The European Federation of Health Product Manufacturers suggest an upper safe level of 800 mg α -tocopherol equivalents. An upper limit for short-term consumption was not established (EHPM 1997).

Summary

106. Vitamin E is a generic designation for a group of eight compounds synthesised by plants. These compounds fall into two classes, tocopherol and tocotrienol, which exhibit the biological antioxidant activity of vitamin E. Their basic structure consists of a hydroxylated ring system (chromanol ring) and an isoprenoid sidechain. Plant oils are the main dietary sources of vitamin E, with meat and dairy products providing only moderate amounts of the daily need.
107. It has been debated whether vitamin E functions solely as a lipid antioxidant, or whether it may also be required for the function of some other critical, but unknown metabolic factor. Information currently available indicates that all its nutritional effects are consistent with its role as a biological antioxidant. In this regard, vitamin E is thought to have a basic functional importance in the maintenance of membrane integrity in virtually all cells of the body.
108. The clinical manifestations of vitamin E deficiency vary considerably between species. In general, however, the targets are the neuromuscular, vascular and reproductive systems. The various signs of vitamin E deficiency are believed to be manifestations of membrane dysfunction, resultant of the oxidative degradation of polyunsaturated membrane phospholipids and/or the disruption of other critical cellular processes.
109. α -Tocopherol is absorbed unchanged from the small intestine by non-saturable, passive diffusion. Tocotrienol esters are first hydrolysed by pancreatic esterase. Absorption appears to mostly occur in the upper and middle thirds of the small intestine. The absorption efficiency of tocopherol and its esters is generally considered to be variable. In human studies, over 24 hours, absorption of α -tocopherol and its acetate ester was in the region of 21 - 86%. In rats given a single bolus of α -tocopherol intraduodenally absorption was reported to be approximately 40%, whereas when α -tocopherol acetate was given as slow continuous infusion into the duodenum, absorption was 65%. At normal levels of intake of the vitamin, it is excreted conjugated with glucuronic acid via the bile in the faeces; less than 1% of vitamin E is excreted in the urine under these circumstances. The urinary metabolites that have been identified are α -tocopheronic acid and α -tocopheronolactone.
110. Vitamin E is one of the least toxic of the vitamins. Animals and humans appear to be able to tolerate high levels of the vitamin (i.e. at least two orders of magnitude above nutritional requirements, e.g. 1000 – 2000 IU/kg diet) without untoward effects. At very high doses, however, vitamin E can produce signs indicative of antagonism with the function of the other fat-soluble vitamins. Thus, animals with hypervitaminosis E have been found to show impaired bone mineralisation, reduced hepatic storage of vitamin A and coagulopathies. In each case, these signs could be corrected with increased dietary supplements of the appropriate vitamin (i.e. vitamins D, A and K, respectively). The antagonism appears to be based at the level of absorption. A number of clinical trials have been conducted and few side

effects have been reported. However, in the ATBC study of Finnish smokers, α -tocopherol treatment was associated with an increase in deaths from haemorrhagic stroke. In the Cambridge Heart Anti-Oxidant trial (CHAOS) a non-significant excess of cardiovascular deaths was found in the treatment groups. The significance and explanation for the latter findings is uncertain. Other large scale epidemiological studies in female nurses and male health professional have not found similar associations.

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ANNEX 1 TO EVM/00/13.REVISED AUG2001

Table 2. Human Supplementation Studies of Vitamin E

Subjects	Dose	Duration	Endpoint/findings	NOAEL/ LOAEL	Comment	Reference
8 Healthy male volunteers	800 IU (mg) dl- α -tocopheryl acetate.	Unstated. Double-blind, Placebo-controlled (DBPC)	Two subjects withdrew from trial on basis of severe fatigue and weakness. Creatinuria and elevated serum creatine kinase observed.	Effects at 800 mg/day	Letter describing drop outs rather than reporting study.	Briggs, 1974
30 Healthy adults	800 IU vitamin E (form unclear)	16 weeks DBPC	Decreased LDL. No Adverse effects reported.	No Effects at 800 IU	Unclear whether side effects specifically sought.	Stampfer <i>et al</i> , 1983
30 elderly patients with stable angina	900 mg (IU)/day dl- α -tocopheryl acetate.	4 months DBPC	Decreased plasma insulin. LDL and triglycerides. No Adverse effects related to treatment reported.	No Effects at 900 IU	Unclear how nature of side effects ascertained.	Paolisso <i>et al</i> , 1995
ATBC study >29,000 male smokers	50 mg α -dl-tocopheryl acetate/day (50 IU) 20 mg/day β -carotene, both α -tocopherol and β -carotene, or placebo DBPC	5-8 years	Lung cancer incidence not affected, but prostate cancer reduced. No effect on total mortality but increased mortality from haemorrhagic stroke (7.8 deaths per 10,000 person years versus 5.2 deaths per 10,000 person years in the no α -tocopherol groups). Deaths from ischaemic stroke and heart disease reduced in the α -tocopherol group. 4% decrease in primary major coronary events and 9% decrease in fatal coronary heart disease in treatment group. Incidence of non fatal myocardial infarction	Effect at 50 IU	Side Effects no reported.	Albanes <i>et al</i> , 1994 Virtamo, <i>et al</i> , 1998

			not affected.			
Cambridge Heart Antioxidant Study (CHAOS). 2002 patients with atherosclerosis	800 IU vitamin E/day for the first 546 patients and 400 IU/day for the remainder (268 or 537 mg free 2R, 4'R, 8'R tocopherol)	510 days (range 3-981) DBPC	Reduction in risk of cardiovascular death + non fatal myocardial infarction (41 events vs 64, RR 0.53, 95%CI 0.34-0.83, p=0.005). Decrease due to decreased MI since, a non-significant excess of cardiovascular deaths found in the treatment group (27 events vs 23, RR 1.18, 95%CI 0.62-2.27, p=0.61).	Effects at 800 and 400 IU	Side Effects not reported.	Stephens <i>et al</i> , 1996
GISSI-Prevenzione trial. 11, 324 patients recovering from MI.	Ig PUFA (n=2836), 300 mg vitamin E (n=2830) both (n= 2830), neither (n=2828)	3.5 years	Combined endpoint death, non-fatal MI, and stroke.	No side effects reported. Vitamin E had no effect on specified end points.	Unclear minor side-effects ascertained.	GISSI-prevenzione Investigators, 1999.
Primary Prevention Project. 4495 people with risk factors for cardiovascular disease.	100 mg/day aspirin (n=2226) or 300 mg/day vitamin E (n=2231)	3.6 years (mean follow up)	Combined efficacy endpoint, cumulative rate of cardiovascular disease, non-fatal myocardial infarction and non-fatal stroke.	No side effects reported. Vitamin E had no effect on specified end points.	Unclear if minor side effects ascertained.	Primary Prevention Project Group, 2001.
HOPE study. 9541 at high risk of cardiovascular events.	ramipril or 400 IU vitamin E (n=4761)	3.6 years (mean follow up)	Combined endpoint, myocardial infarction stroke and death from cardiovascular causes. Secondary outcomes also considered	No side effects reported. Vitamin E had no effect on specified end points.	Unclear if minor side effects ascertained.	Yusuf <i>et al.</i> , 2000
MRC/BHF study	600 mg/day α -tocopherol, 250 mg vitamin C, 20 mg β -carotene or	5 years	All cause mortality, vascular and non-vascular mortality, non-fatal MI, non-fatal or fatal stroke, cancer incidence, hospitalisation for non-vascular	No serious side effects observed.	Unclear if minor side effects ascertained.	Heart Protection Study Collaborative Group, 2002.

	placebo in 20,536 "high risk adults"		causes.			
10 Healthy volunteers	400 mg/day	4 weeks	Blood and plasma viscosity, red cell deformability, red cell aggregation, colloid osmotic pressure of plasma, blood count, total lipids and α -tocopherol measured. Significant increase in red cell deformability and α -tocopherol levels measured.		Effects at 400 mg/day	Ernst and Matrai, 1985
Healthy adults	1000 mg vitamin E	6 weeks	Sensitivity to oxidative DNA damage assessed. No Adverse effects reported.	No Effects at 1000 mg/day	Unclear whether side effects specifically sought.	Brennan <i>et al</i> , 2000
Healthy adults	0 or 400 (IU) vitamin E		DNA repair activity assessed. No Adverse effects reported.	No Effects at 400 IU/day	Unclear whether side effects specifically sought.	Huang <i>et al</i> , 2000
31 Healthy adults	0, 15, 60 or 200 mg/day (IU) dl- α -tocopherol acetate	4 weeks DBPC	DNA repair activity assessed. No Adverse effects reported.	No Effects at 200 mg/day (IU)	Unclear whether side effects specifically sought.	Hu <i>et al</i> , 1996
28 Diabetic subjects	0 or 1200 IU (1632 mg)/day RRR- α -tocopherol	8 weeks	Effect on protein glycation and LDL assessed. No Adverse effects reported.	No Effects at 1632 mg/day (1200IU)	Unclear whether side effects specifically sought.	Fuller <i>et al</i> , 1996
36 Diabetic and 9 non diabetic subjects	0 or 1,8000 IU vitamin E	4 months DBPC crossover	Retinal blood flow and creatinine normalised by treatment. Dizziness, headache, fatigue, breast pain and diarrhea reported. No association with treatment. One patient dropped out due to thyroid condition.			Bursell <i>et al</i> , 1999
48 Healthy male volunteers	0, 60, 200, 400, 800 or 1200 IU α -tocopherol	8 weeks, randomized single-blind study	Effect on LDL and lipid peroxidation assessed. No Adverse effects experienced.	No Effects at 1200 IU/day	.	Jialal <i>et al</i> , 1995

	(form uncertain)					
48 patients with stable angina	0 or 1600 IU α -tocopherol	6 months, DBPC crossover	No differences in cardiac parameters. The subjects were questioned regarding possible side effects and underwent periodic urinalysis, blood chemistry analysis and complete blood count as well as measures of prothrombin time, a chest X ray and ECG. No deleterious side effects were observed. Slightly increased incidence of gastrointestinal disturbance during the placebo phase. There was no exacerbation of hypertension, congestive heart failure or skeletal muscle complaints could be attributed to treatment. No differences were found in the various clinical chemistry studies.	No effects at 1600 IU/day	.	Gillilan <i>et al</i> , 1977
Part 1 40 patients Part 2. 15 patients already taking vitamin E supplements	Part 1. 0 or 3200 IU α -tocopherol succinate Part 2. Dose unclear	Part 1. 9 weeks. DBPC Part 2. Duration unclear	No differences in cardiac parameters. Some gastrointestinal disturbance in treatment group.	Some effects at 3200 IU/day		Anderson, <i>et al</i> , 1974
80 Healthy older subjects	0 or 800 (IU) RRR - α -tocopherol	4 months	A wide range of clinical, haematological and other parameters investigated. Including creatine, prothrombin times and blood counts	No adverse effects occurred.		Meydani, <i>et al</i> , 1998
Elderly subjects. 369 supplement users, 1,861 non-users.	Unknown	2 years observation	Vitamin E users more likely to complain of shortness of breath and angina. Clinical parameters	N/A	Supplement use assessed by questionnaire	Hale <i>et al</i> , 1986.

			unaffected by treatment with the exception of SGOT which was elevated in male supplement users.			
25 patients on warfarin therapy	0, 800 or 1200 IU	1 month	International normalised ratio (bleeding time)	No adverse effects occurred		Kim and White, 1996

Table 3. Animal Toxicity

Species	Dose	Duration	Endpoint/findings	NOAEL/LOAEL	Comment	Reference
<i>Subchronic Toxicity</i>						
Rats (10/sex/group)	0,125, 500 or 2000 mg/kg bw/ α -tocopherol acetate/day by gavage	13 weeks	Increased relative liver weights. Deaths and morbidity at top dose, increased prothrombin and activated thromboplastin times. Decreased haematocrit, haemoglobin levels. Reticulocytosis.	125 mg/kg bw/day		Abdo <i>et al</i> , 1986.
Rats (15sex/group)	0,0.002, 0.02 or 2% d-a-tocopherol poly(ethylene glycol) in the diet. Quantities ingested 0.32-0.48, 31.5-45.6 and 316.8-443.1 mg/rat respectively.	90 days	No effect on body or organ weights, behaviour, appearance, haematology, clinical chemistry or pathology.	2% in diet.	Pathology conducted on control and high dose only.	Krasavage and Terhaar, 1977.
Rats	4 g/kg bw/day	2 months		50g/kg bw/day "Can be tolerated"		Demole <i>et al</i> , 1939
Rats	100mg/kg bw/day α -tocopherol	19 weeks	Increased phosphorus metabolism.	NOAEL = 10 mg/kg bw/day		Weissburger and Harris, 1943
Rats	150mg week α -tocopherol	20-27 weeks	Fatty changes in liver, intimal sclerosis of the aorta.	LOAEL = 10 mg/kg bw/day	Vegetable oil concentrate used so other components could have been responsible for findings.	Marx <i>et al</i> , 1947.
Weanling rats	α -tocopherol at 35, 875, 1750, 3500 or 35,000 mg/kg in diet	8 weeks	Decreased feed and protein efficiency. Increased SGPT levels.	NOAEL 175 mg/kg bw/day	The study is only published as an abstract.	Dysmsza and Park, 1975.

	(approximately 3.5 –3500 mg/kg bw /day)					
Mice	50 g/kg bw/day	2 months		50g/kg bw/day “Can be tolerated”		Demole <i>et al</i> , 1939.
Chicks	2200 IU/day dl- α -tocopheryl acetate/kg	3-8 weeks	Reduced growth rate, prolonged prothrombin time, depressed thyroid hypertrophy in response to thiouracil.	NOAEL =1000 IU		March <i>et al</i> , 1973
Chronic Toxicity						
Weanling female Wistar rats	α -tocopherol at 0, 25, 250, 2500, 10,000 or 25,000 mg/kg in diet (approximately 2.5 –2,500 mg/kg bw /day)	8 and 16 months	Decreased body weight gain, increased relative heart and spleen weights Increased alkaline phosphatase levels.	NOAEL 250 mg/kg bw/day	No Histological examinations reported.	Yang and Desai, 1977
CD Rats (50/sex/group)	α -tocopherol in diet to give doses of 500,1000 or 2000 mg/kg bw /day)	104 weeks	Vitamin K supplementation needed in all groups due to haemorrhages. A trend towards fewer mammary tumours in females. Increased ALT levels in early part of study.	LOAEL 500 mg/kg bw/day		Wheldon <i>et al</i> , 1983
Reproductive Toxicity						
Rats (15/sex/group)	0,0.002, 0.02 or 2% d- α -tocopherol poly(ethylene glycol) in the diet.	90 days prior to mating and offspring thereafter to 5 weeks post-weaning.	No effect on a range of reproductive and developmental parameters	NOAEL 2% in diet		Krasavage and Terhaar, 1977
Weanling rats	α -tocopherol at 35, 875, 1750, 3500 or 35,000 mg/kg in diet (approximately 3.5 –3500 mg/kg	8 weeks	Decreased number of live pups in top dose group.	NOAEL 3500 mg/kg in diet (350 mg/kg bw/day)	The study is only published as an abstract.	Dysmsza and Park, 1975.

	bw /day)					
Rats (15/sex/group)	0,0.002, 0.02 or 2% d- α -tocopherol poly(ethylene glycol) in the diet.	Days 6-16 of gestation	No dose-related abnormalities apparent.	NOAEL 2% in diet		Krasavage and Terhaar, 1977
Rats	22.5 to 2252 mg/kg bw α -tocopherol acetate	During pregnancy and lactation	No teratogenic effects observed, litter size, weight and survival unaffected. Delayed eye opening in several newborns in 500mg/group but not significant.	NOAEL = 2252 mg/kg bw/day		Martin and Hurley, 1977
ICR mice	591 IU d- α -tocopherol/day by gavage	Days 7-11 of gestation	1 malformation in 91 offspring from treated litter, exencephaly, open eye and micrognathia not seen in strain before.	NOAEL 2% in diet		Hook <i>et al</i> , 1977

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INTAKES OF VITAMIN E³ FROM FOOD AND SUPPLEMENTS

The data presented on intakes of the above are obtained from dietary surveys of specific population age groups in Britain carried out over the last 15 years⁴⁵⁶⁷⁸. In each survey food consumption data were collected by means of a dietary record (usually weighed) kept for 4 or 7 consecutive days. Nutrient intakes were calculated using a set of nutrient composition data contemporaneous with the time of the survey. Therefore some apparent differences in intakes between population age groups may be due to changes in the nutrient composition data and reflect changes in the nutrient composition of manufactured foods over time.

Total intakes of vitamin E

Table 1 provides information on the absolute intakes of vitamin E by the British population, classified by age and sex. Mean and median intake, and the upper and lower end of the intake distribution (defined as upper and lower 2.5 percentiles, respectively) are given.

Average intake of vitamin E from food was lowest for infants and pre-school children and highest in males aged 15 to 74 years. Intakes of vitamin E from food increased significantly with age for pre-school children and young people aged 4 to 18 years, and decreased significantly with age for those aged 65 years and over free-living in the community. Intakes from food only, and from food and supplements at the 97.5%ile were about 2-3 times the median in all groups except for females aged 50 to 74 years, where intakes at the 97.5%ile from food and supplements were about 5-6 times the median.

Table 2 provides information on Vitamin E intakes adjusted for body weight classified by age and sex. Vitamin E intakes adjusted for body weight showed a trend to decrease with age for pre-school children, young people and males aged 65 years and over free-living in the community, and increase with age for females aged 16-64 years due to the higher consumption of supplements in the older females.

³ α -tocopherol equivalents

⁴ Food and nutrient intakes of British infants. 1986

⁵ National Diet and Nutrition Survey of children aged 1½-4½ years. 1992/3

⁶ National Diet and Nutrition Survey of young people aged 4-18 years. 1997/8

⁷ Dietary and nutritional survey of British adults. 1986/7

⁸ National Diet and Nutrition Survey of people aged 65 years and over. 1994/5

Sources of vitamin E in the diet

Table 3 indicates the contribution made by different types of food to average intakes of vitamin E by young people aged 15-18 years. This dataset was collected in 1997 and so most closely reflects current eating habits and fortification practices.

The main source of vitamin E in this age group is vegetables, potatoes and savoury snacks (namely potato chips, and crisps and savoury snacks, derived from the oils used for frying or in the manufacture of these foods). This is followed by fat spreads (the majority of which is from polyunsaturated reduced fat spreads), then cereals and cereal products (partly from the vegetable fats in cereal based foods such as biscuits, buns, cakes, pastries, fruit pies and cereal based puddings).

The main sources of vitamin E were similar for those aged 1½ to 4½ years, however the main source of vitamin E for older people was fat spreads, and for infants was infant formula and milk.

Vitamin E is added as a fortificant to some breakfast cereals, and as antioxidant preservative to a range of foods including polyunsaturated fat spreads.

Vitamin E intakes from supplements

For adults and free-living older people, dietary supplements containing vitamin E provided 16% and 25% respectively of population average intakes of vitamin E. Dietary supplements containing vitamin E provided 2% of population average intakes for both toddlers and young people. Equivalent data for infants are not available. Of course, the proportion of intake from supplements is much higher if supplement consumers are considered separately.

Table 4 shows the number of consumers of dietary supplements containing vitamin E in each age group, together with the mean, median and range of intakes of vitamin E from supplements for those who consumed them. The highest prevalence of vitamin E supplement use was in those older people free-living in the community. Within this group, 12% of males and 15% of females took vitamin supplements containing vitamin E. Only a small proportion of pre-school children, young people, adults and older people living in institutions took vitamin E supplements.

The range of intakes from supplements was wide with maximum intakes from this source at around 600mg per day by male adults and female older people free-living in the community. These high intakes of vitamin E from supplements were due in part to the use of high dose vitamin E tablets, multivitamin and mineral tablets, cod liver oil and evening primrose oil preparations.

It should be borne in mind that the data for adults aged 16-64 years was collected in 1986/87 and use of supplements may have changed since then.

Diet and Nutrition Surveys Branch
Nutrition Division
Food Standards Agency

Table 1: Total intakes of Vitamin E

Age/sex	Absolute Vitamin E intake (mg/day)							
	Food Only				Food and Supplements			
	2.5% ile	Mean	Median	97.5%ile	2.5% ile	Mean	Median	97.5%ile
Infants (1986) 6-12mths/M&F	1.4	4.0	3.2	9.9	*	*	*	*
Pre-school children 1½-2½ yrs/M/F	1.4	3.8	3.5	8.5	1.4	3.9	3.6	9.0
2½-3½ yrs/M/F	1.8	4.4	4.1	8.5	1.8	4.5	4.1	9.6
3½-4½ yrs/M	2.1	5.0	4.5	10.0	2.1	5.1	4.6	10.7
3½-4½ yrs/F	2.0	4.5	4.1	9.2	2.0	4.6	4.3	10.6
Young people (1997/8) 4-6 yrs/M	3.3	6.6	6.2	12.4	3.3	7.2	6.3	14.7
4-6 yrs/F	3.2	6.3	5.7	11.2	3.2	6.6	6.0	16.3
7-10 yrs/M	4.0	8.1	7.5	15.8	4.0	8.3	7.5	18.3
7-10 yrs/F	3.8	7.6	7.1	15.3	3.8	7.8	7.2	15.7
11-14 yrs/M	3.5	9.1	8.6	16.5	3.5	9.1	8.6	16.5
11-14 yrs/F	3.8	8.1	7.5	15.0	3.8	8.2	7.6	15.5
15-18 yrs/M	4.4	10.3	9.3	19.6	4.4	10.3	9.3	19.6
15-18 yrs/F	3.3	8.1	7.2	16.1	3.3	8.6	7.5	16.1
Adults (1986/7) 16-24 yrs/M	3.5	9.7	9.2	18.6	4.0	10.7	9.2	18.7
16-24 yrs/F	2.4	6.8	6.1	13.8	2.4	7.0	6.1	16.9
25-34 yrs/M	3.8	10.2	9.6	18.2	3.8	13.0	9.6	20.3
25-34 yrs/F	2.1	7.3	7.0	15.1	2.1	8.1	7.0	20.0
35-49 yrs/M	3.8	10.4	9.4	24.0	3.8	12.2	9.4	27.0
35-49 yrs/F	3.0	7.6	7.0	16.1	3.2	8.5	7.1	19.7
50-64 yrs/M	2.6	9.2	8.8	18.1	2.6	10.6	8.9	20.7
50-64 yrs/F	2.4	7.0	6.6	14.1	2.5	10.2	6.8	43.9
Older people free-living in the community (1994/5)								

65-74yrs/M	2.7	9.3	8.2	21.1	2.7	10.7	8.4	25.8
65-74yrs/F ⁹	1.8	6.9	6.0	16.0	1.8	11.0 (11.9)	6.4	32.3
75-84 yrs/M	2.8	8.7	7.4	22.5	2.8	9.2	7.5	22.7
75-84 yrs/F ⁷	1.8	6.9	5.8	17.9	1.8	10.2 (11.6)	5.9	19.6
85 and over/M	2.1	6.7	5.7	16.3	2.1	7.8	5.8	17.1
85 and over/F	1.5	5.8	4.8	14.4	1.7	7.6	5.2	18.1
Older people living in institutions (1994/5)								
65-84 yrs/M	2.5	7.7	6.2	20.2	2.5	7.7	6.2	20.2
65-84 yrs/F	2.2	6.9	5.7	16.9	2.3	7.0	5.9	16.9
85 and over/M	2.2	7.9	6.9	22.6	2.2	7.9	6.9	22.6
85 and over/F	2.1	6.3	5.4	14.3	2.1	6.4	5.4	14.3

* Data unavailable

Table 2: Bodyweight adjusted vitamin E intake

Age/sex	Bodyweight adjusted vitamin E intake (mg/kg bwt /day) ¹⁰		
	<i>intakes from food and supplements</i>		
	Mean	Median	97.5%ile
Infants (1986)¹¹			
6-12mths/M&F	0.39	0.30	1.10
Pre-school children (1992/3)			
1½-2½ yrs/M&F	0.323	0.293	0.733
2½-3½ yrs/M&F	0.309	0.281	0.658
3½-4½ yrs/M	0.308	0.276	0.617
3½-4½ yrs/F	0.287	0.258	0.618
Young people (1997/8)			

⁹ Vitamin E intake values including supplements for 2 women in the 65-74 and 75-84 year age groups were very high and were trimmed. The values that were trimmed were 608.31 and 621.00mg/day. Mean intakes of the groups before trimming are given in brackets.

¹⁰ Body weights measured for each subject for all age groups except infants aged 6-12 months where reported body weights were used.

¹¹ Intakes for infants aged 6-12 months are from food only.

4-6 yrs/M	0.340	0.301	0.690
4-6 yrs/F	0.325	0.303	0.624
7-10 yrs/M	0.282	0.255	0.614
7-10 yrs/F	0.252	0.230	0.573
11-14 yrs/M	0.200	0.196	0.369
11-14 yrs/F	0.176	0.165	0.359
15-18 yrs/M	0.159	0.144	0.304
15-18 yrs/F	0.148	0.124	0.294
<i>Adults (1986/7)</i>			
16-24 yrs/M	0.155	0.134	0.273
16-24 yrs/F	0.119	0.106	0.261
25-34 yrs/M	0.174	0.126	0.289
25-34 yrs/F	0.136	0.111	0.302
35-49 yrs/M	0.162	0.122	0.398
35-49 yrs/F	0.136	0.112	0.320
50-64 yrs/M	0.139	0.110	0.262
50-64 yrs/F	0.164	0.109	0.688
<i>Older people free-living in the community (1994/5)</i>			
65-74 yrs/M	0.141	0.111	0.326
65-74 yrs/F	0.181	0.100	0.439
75-84 yrs/M	0.127	0.106	0.312
75-84 yrs/F	0.194	0.093	0.306
85 and over/M	0.111	0.088	0.226
85 and over/F	0.126	0.086	0.336
<i>Older people living in institutions (1994/5)</i>			
65-84 yrs/M	0.114	0.095	0.273
65-84 yrs/F	0.119	0.105	0.220
85 and over/M	0.118	0.103	0.314
85 and over/F	0.109	0.095	0.272

Table 3¹²: Sources of vitamin E in the diet

Food Type	Contribution of food types to average daily intake of vitamin E	
	mg/day	% of total
Cereal and cereal products	1.5	16
Milk and milk products	0.3	3
Egg and egg dishes	0.2	2
Fat spreads	1.9	21
<i>- of which reduced fat spreads</i>	<i>1.1</i>	<i>12</i>
<i>(polyunsaturated)</i>		
Meat and meat products	1.0	11
Fish and fish dishes	0.3	3
Vegetables, potatoes and savoury snacks	2.7	29
<i>- of which roast or fried potatoes & chips</i>	<i>0.9</i>	<i>10</i>
<i>- of which crisps & savoury snacks</i>	<i>0.8</i>	<i>9</i>
Fruits and nuts	0.2	2
Sugar, confectionery and preserves	0.5	6
<i>- of which chocolate confectionery</i>	<i>0.5</i>	<i>6</i>
Beverages	0.1	1
Miscellaneous	0.5	6
Total intake from food	9.2	100
<i>Intake from dietary supplements</i>	<i>0.3</i>	<i>3</i>
Total intake from food and supplements	9.5	100

¹² NDNS: young people aged 4-18 years. 1997/8. 15-18 year group

Table 4: Vitamin E intake from supplements

<i>Age/sex</i>	Consumers of vitamin E supplements		Vitamin E intake from supplements (consumers only) (mg/day)		
	<i>Number</i>	<i>%</i>	<i>Mean</i>	<i>Median</i>	<i>Range</i>
<i>Infants (1986)</i> 6-12 mths/M&F	*	*	*	*	*
<i>Pre-school children (1992/3)</i> 1½-4½ yrs/M&F	63	4	3.2	3	0.3 - 9.0
<i>Young people (1997/8)</i> 4-6 yrs/M&F	15	4	10.5	6.3	0.1 - 42.9
7-10 yrs/M&F	17	4	5.1	4.4	0.1 - 10.0
11-14 yrs/M	2	1	0.7	0.7	0.7 - 0.8
11-14 yrs/F	4	2	5.5	4.6	2.0 - 11.4
15-18 yrs/M	7	4	2.2	1.3	0.3 - 7.1
15-18 yrs/F	9	4	12.9	1.8	0 - 95.7
<i>Adults (1986/7)</i> 16-64 yrs/M	47	4	40.7	3.6	0.3 - 600.0
16-64 yrs/F	90	8	17	3.6	0 - 200.0
<i>Older people free-living in the community (1994/5)</i> 65 and over/M	73	12	8.9	2.0	0.1- 100.5
65 and over/F	99	15	28.4	4.2	0.1 - 610.8
<i>Older people living in institutions (1994/5)</i> 65 and over/M	4	2	2.0	0.6	0.5 - 6.4
65 and over/F	7	3	1.9	0.7	0.3 - 5.0

* Data unavailable

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Vitamin E (α - Tocopherol Equivalent, TE) : Summary table of selected nutrition related information and existing guidance on regulations

Unit of usage	mg/day		Mg/100 kcal
	Male	Female	
UK DRV ¹³ for adults (19-50+) <i>Safe Intake</i>	Above 4mg/day dependent on PUFA intake	Above 3mg/day dependent on PUFA intake	
<i>Mean adult UK dietary intake from food (all sources)</i>			
Adults 16-64 years ¹⁴	9.9 (11.7)	7.2 (8.6)	
Adults 65 years and over ¹⁵			
free living	9.0 (10.1)	7.7 (10.3)	
institutionalised	7.8 (7.8)	6.9 (6.9)	
EU labelling RDA ¹⁶	10		
Supplemental doses	2 – 670 mg/unit		

¹³ Committee on Medical Aspects of Food and Nutrition Policy (1991). Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects 41. London: HMSO.

¹⁴ Dietary and nutritional survey of British adults. 1986/7

¹⁵ National Diet and Nutrition Survey of people aged 65 years and over. 1994/5

¹⁶ The Food Labelling Regulations 1996

Regulations		
Infant formula ¹⁷		At least 0.5 mg TE/100 kcal*
Follow-on formula		At least 0.5 mg TE/100 kcal*
Weight reduction ¹⁸ whole daily diet replacement meal replacement	10 3 mg/meal	
<i>Maximum total safe daily intake</i>		
COMA 1991 ¹	Up to 3200	
EHPM 1997 ¹⁹	Upper safe level 800 mg α -TE	

* At least 0.5mg TE/g of polyunsaturated fatty acids (PUFA's) expressed as linoleic acid per 100 available kcal.

¹⁷ The Infant Formula and Follow-on Formula Regulations 1995

¹⁸ The Foods Intended for Use in Energy Restricted Diets for Weight Reduction Regulations 1997.

¹⁹ Vitamins and Minerals A Scientific Evaluation of the Range of Safe Intakes. European Federation of Health Product Manufacturers 1997.

