Bioactivity of vitamin E

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More than 80 years after the discovery of the essentiality of vitamin E for mammals, the molecular basis of its action is still an enigma. From the eight different forms of vitamin E, only \( \alpha \)-tocopherol is retained in the body. This is in part due to the specific selection of \( \textit{RRR} \)-\( \alpha \)-tocopherol by the \( \alpha \)-tocopherol transfer protein and in part by its low rate of degradation and elimination compared with the other vitamers. Since the tocopherols have comparable antioxidant properties and some tocotrienols are even more effective in scavenging radicals, the antioxidant capacity cannot be the explanation for its essentiality, at least not the only one. In the last decade, a high number of so-called novel functions of almost all forms of vitamin E have been described, including regulation of cellular signalling and gene expression. \( \alpha \)-Tocopherol appears to be most involved in gene regulation, whereas \( \gamma \)-tocopherol appears to be highly effective in preventing cancer-related processes. Tocotrienols appear to be effective in amelioration of neurodegeneration. Most of the novel functions of individual forms of vitamin E have been demonstrated \textit{in vitro} only and require \textit{in vivo} confirmation. The distinct bioactivities of the various vitamers are discussed, considering their metabolism and the potential functions of metabolites.

Vitamin E: Bioactivity: \( \alpha \)-Tocopherol: Vitamers: Antioxidants

Introduction

Vitamin E is a family of eight compounds, \( \alpha \)-, \( \beta \)-, \( \gamma \)- and \( \delta \)-tocopherol and \( \alpha \)-, \( \beta \)-, \( \gamma \)- and \( \delta \)-tocotrienol (Fig. 1), with \( \alpha \)-tocopherol being the predominant form in mammals (for reviews, see Burton & Traber, 1990; Brigelius-Flohé & Traber, 1999). The tocopherols have three chiral centres in the natural forms are present exclusively as \( \textit{RRR} \)-isomers. Synthetic \( \alpha \)-tocopherol is a racemic mixture containing all possible combinations of \( R \) and \( S \) stereoisomers. Vitamin E has been detected as a factor capable of preventing the resorption of fetuses in rats and is now commonly used for the breeding of farm animals. The need for vitamin E for the successful reproduction of rats is taken as a measure for the bioactivity of individual forms of vitamin E. In this fetal resorption--gestation assay, \( \alpha \)-tocopherol has the highest activity (100 %), followed by \( \beta \)-tocopherol (57 %), \( \gamma \)-tocopherol (37 %) and \( \delta \)-tocopherol (1-4 %). The activities of \( \alpha \)-tocotrienol and \( \beta \)-tocotrienol are 30 and 5 % of that of \( \alpha \)-tocopherol (for a review, see Azzi & Stocker, 2000). Also the bioactivity of natural and synthetic \( \alpha \)-tocopherol is different. Compared with \( \textit{RRR} \)-\( \alpha \)-tocopherol, \( \textit{RRS} \) has 90 %, \( \textit{RSS} \) 73 %, \( \textit{SSS} \) 60 %, \( \textit{RSR} \) 57 %, \( \textit{SRR} \) 37 %, \( \textit{SRS} \) 31 % and \( \textit{SSR} \) 21 % activity in the rat fetal resorption--gestation test (Weiser & Vecchi, 1982).

The reason for the high efficacy of \( \textit{RRR} \)-\( \alpha \)-tocopherol is obviously its preferential retention in the organism. Whereas in the intestine all forms of vitamin E are absorbed without discrimination, in the liver only \( \alpha \)-tocopherol is sorted out by the \( \alpha \)-tocopherol transfer protein (\( \alpha \)-TTP) for incorporation into VLDL and subsequent distribution to peripheral tissues (for a review, see Traber & Arai, 1999). \( \alpha \)-TTP specifically binds \( \alpha \)-tocopherol. The affinity to other forms of vitamin E is lower, being 38 % for \( \beta \)-, 9 % for \( \gamma \)-, and 2 % for \( \delta \)-tocopherol. The affinity for the synthetic \( \textit{SRR} \) form is 11 % and for \( \alpha \)-tocotrienol 12 % compared with that for \( \alpha \)-tocopherol (Hosomi et al. 1997). The crucial role of \( \alpha \)-TTP for \( \alpha \)-tocopherol distribution is demonstrated by the infertility of \( \alpha \)-TTP knockout mice (Jishage et al. 2001) and the severe general vitamin E deficiency with characteristic neurological disorders and ataxia in patients with a mutation in the \( \alpha \)-TTP gene (Ben Hamida et al. 1993; Ouahchi et al. 1995; Hentati et al. 1996). Thus, one of the reasons for the preference for \( \alpha \)-tocopherol is its selective recognition by \( \alpha \)-TTP. A second reason for the high bioactivity of \( \alpha \)-tocopherol is its comparatively low metabolic degradation. Tocopherols and tocotrienols are metabolised by side-chain degradation. All forms of vitamin E are degraded along the same pathway; their metabolic rates, however, differ greatly. This will definitely influence the bioactivities of individual forms of vitamin E.

Abbreviations: CEHC, carboxyethyl hydroxychroman; COX, cyclo-oxygenase; CYP, cytochrome P450; IC\textsubscript{50}, 50 % inhibitory concentration; PKC, protein kinase C; PXR, pregnane X receptor; \( \alpha \)-TTP, \( \alpha \)-tocopherol transfer protein.

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Over the last decade distinct novel functions have been described for different forms of vitamin E. They may be relevant to cancer, neurodegeneration and inflammation. Underlying mechanisms – as far as elucidated – are not related to the antioxidant capacity of tocopherols and tocotrienols. These functions will be summarised and discussed in the present review in respect to the metabolic rate of individual forms of vitamin E and, accordingly, the relevance of the novel function to human health.

**Metabolism of vitamin E**

Vitamin E is not metabolically inert. All forms of vitamin E are degraded by the same mechanism, an initial ω-hydroxylation followed by β-oxidation (for reviews, see Brigelius-Flohé *et al.* 2002b; Pfuger *et al.* 2004). ω-Hydroxylation is catalysed by cytochrome P450 enzymes (CYP), of which CYP3A4 (Parker *et al.* 2000; Birringer *et al.* 2001) and CYP4F2 (Sontag & Parker, 2002) are the most likely candidates. The final products of all forms are the respective carboxyethyl hydroxychromans (CEHC) (Fig. 1). CEHC are conjugated with glucuronic acid or sulfate and eliminated in the urine. Precursors of CEHC, carboxymethyl hydroxychromans, can also be found in the urine, although to a much lesser extent (Parker & Swanson, 2000; Schuelke *et al.* 2000) (Fig. 1). Considering the pathway of degradation reveals that vitamin E is metabolised like xenobiotics. First, a functional group is introduced via the phase 1 enzyme(s) CYP3A4 or CYP4F2, then β-oxidation follows. The degradation product is conjugated by phase 2 enzymes such as UDP-glucuronosyltransferases or sulfotransferases, and the conjugate is finally eliminated via the bile or urine. Whether elimination requires phase 3 transporters such as MDR-1 or MRP-2 remains to be investigated.

To evaluate the metabolic fate of the different vitamers in *vivo*, concentrations of metabolites have been measured in human urine (Swanson *et al.* 1999; Schuelke *et al.* 2000), human plasma (Stahl *et al.* 1999; Radosavac *et al.* 2002; Galli *et al.* 2003, 2004) and rat bile (Hattori *et al.* 2000; Kiyose *et al.* 2001). Consistently 1–3% of the ingested α-tocopherol can be found as α-CEHC in human urine (Schuelke *et al.* 2000). In contrast, γ-tocopherol has been calculated to be metabolised to γ-CEHC up to 50%.
(Swanson et al. 1999). A recent study reported that this percentage can even be higher (Galli et al. 2002). Whereas basal plasma levels of γ-tocopherol in eight healthy volunteers were fifteen times lower than those of α-tocopherol (2.03 ± 3.16 μmol/l), the levels of plasma γ-CEHC were at least ten times higher than that of α-CEHC (160 ± 12.5 μmol/l). From an oral intake of 100 mg 2H-labelled γ-tocopherol, 18 mg was transferred into the blood, confirming limited intestinal absorption of high dosages. Interestingly, 2H-labelled γ-CEHC concentration in plasma also accounted for 18 mg γ-tocopherol equivalents, indicating an almost quantitative degradation (Galli et al. 2002). However, only one-third of the plasma γ-CEHC appeared in the urine, which suggests alternative routes for γ-CEHC excretion. The urinary amount of γ-CEHC (6.5 mg γ-tocopherol equivalents) accounts for 6.5% of the ingested or 36% of the absorbed parent compound. Assuming that the same situation holds true for γ-tocotrienol, the 4–6% of the applied γ-tocotrienol that was recovered as γ-CEHC in the urine (Lodge et al. 2001) would equally indicate an almost 100% degradation of γ-tocotrienol, as discussed by Galli et al. (2002). Such a high degradation of γ-tocopherol could not be confirmed recently. Leonard et al. (2005b) reported that only 1% of the dose is eliminated in the urine. These authors also suggested alternative routes for γ-CEHC excretion. Interestingly, however, women produced almost twice the amount of γ-CEHC from 2H-labelled γ-tocopherol than men. The explanation was offered that women metabolise certain CYP3A4 substrates faster, which would make them better equipped to handle xenobiotics. This implies that γ-tocopherol is, indeed, considered to be foreign and women might profit less from an enhanced γ-tocopherol intake.

Metabolism of α-tocotrienol has been investigated in cell culture (Pfluger et al. 2004). In HepG2 cells, α-tocotrienol yielded fourteen times the amount of α-CEHC than α-tocopherol within 72 h. Surprisingly, however, the precursor, α-carboxymethylbutyl hydroxychroman, was about seventy-five times higher with α-tocotrienol than with α-tocopherol. If this also holds true in vivo, the low amount of α-tocotrienol that was determined as α-CEHC in the urine (1–2% of the applied dosage) (Lodge et al. 2001) would not reflect the actual metabolic rate and underestimate the metabolism of α-tocotrienol. Clearly, an excretion of CEHC and precursors via the bile has to be taken into account. More detailed investigations of metabolic rates and routes are required for all of the vitamins, since a satisfactory balance has not yet been established for any of them. It can only be stated with certainty that the available studies disclose a dramatically faster degradation of γ- than of α-tocopherol, which recently has also been corroborated by measuring, for the first time, liver CEHC concentrations in rats (Leonard et al. 2005a).

The metabolism of a vitamin by pathways, which are usually engaged in the detoxification of xenobiotics, was intriguing and provoked the question if, under certain circumstances, vitamin E is considered as ‘foreign’. Like many xenobiotics, vitamin E induced endogenous CYP3A in cultured cells (Landes et al. 2003) and in mice (Kluth et al. 2005; Traber et al. 2005). Induction of CYP3A forms is mediated by the nuclear pregnane X receptor (PXR) (Lehmann et al. 1998), which is described as a xenobiotic sensor for lipophilic compounds with discrete polarity (Watkins et al. 2001). Vitamin E meets the characteristics of such PXR ligands and, indeed, the activation of a PXR-driven reporter gene by different forms of vitamin E could be demonstrated (Landes et al. 2003). Tocotrienols displayed the highest in vitro activity followed by δ- and α-tocopherol, while α-tocopherol proved the more potent inducer in vivo (see later).

These findings imply that at least some forms of vitamin E may induce their own metabolism and may also affect the metabolism of other CYP substrates. The consequences may be both beneficial and detrimental: maintenance of an optimum xenobiotic metabolising system may protect against harmful food ingredients and environmental poisons, but the induction of this system may also weaken the therapeutic efficacy of essential drugs, as has been discussed elsewhere (Brigelius-Flohé, 2003, 2005; Traber, 2004). The findings also imply that most forms of vitamin E are eliminated like xenobiotics even at low intake, which raises the question why nature decided to degrade the tocotrienols, γ- and δ-tocopherol but not α-tocopherol.

Functions of vitamin E

The antioxidative property of vitamin E was already recognised in the early 1930s. Since then, it has been classified as the major lipid-soluble antioxidant which protects lipids and membranes from oxidative damage in vitro and in vivo (Tappel & Zalkin, 1960; Burton & Ingold, 1981; Burton et al. 1982). Antioxidants in their proper definition are compounds which react with free radicals and, thus, interrupt free radical chain reactions. The respective functional group in the vitamin E molecule is the hydroxyllic group at position 6 in the chroman ring (Fig. 1). This group is characteristic for all forms of vitamin E and, therefore, they can all react as antioxidants, in a test-tube at least. When tested with organic peroxyl radicals as oxidising partners, the order of the antioxidant potential of tocopherols is α- > β- ≈ γ- > δ-tocopherol (Burton & Ingold, 1981). This reflects the order of bioactivity estimated in the fetal resorption–gestation assay, but by no means in quantitative terms. Also α-tocotrienol even has an up to sixty times higher antioxidant activity against Fe3+/ascorbate- and Fe3+/NADPH-induced lipid peroxidation in rat liver microsomal membranes than α-tocopherol (Serbinova et al. 1991). This again does not correlate with its activity in the conventional bioassays and further demonstrates that the in vitro antioxidant activity does not allow conclusions in respect to the in vivo effect. This discrepancy has shed considerable doubt on the prevailing view that the antioxidant property of vitamin E is the real basis of its biological function (for reviews, see Azzi & Stocker, 2000; Munteanu et al. 2004; Zingg & Azzi, 2004). The impact of the current controversy is underscored by recent reports on clinical trials that disprove any efficacy of vitamin E or other antioxidants in preventing CVD, cancer or other disorders believed to result from oxidative stress (Heart Protection Study Collaborative Group, 2002; Genkinger et al. 2004; Lonn et al. 2005; Poston et al. 2006; for reviews, see Brigelius-Flohé et al. 2002a; Upston et al. 2004).
et al. 2003; Stocker & Keaney, 2004; Pham & Plakogiannis, 2005). Even harmful effects of vitamin E have been suggested from meta-analyses of clinical trials (Vivekananthan et al. 2003; Miller et al. 2005). Thus, to understand the real biological role of vitamin E we have to look beyond free radical biochemistry.

**α-Tocopherol**

The key observation that initiated the search for novel functions of vitamin E was the specific inhibition of smooth muscle cell proliferation by α- but not β-tocopherol (Boscoboinik et al. 1991). The underlying mechanism was identified as an inhibitory dephosphorylation of protein kinase C (PKC) that is catalysed by an α-tocopherol-stimulated phosphoprotein phosphatase (Tasinato et al. 1995; Ricciarelli et al. 1998). α-Tocopherol was then shown to inhibit key events in inflammatory signalling, such as platelet aggregation, release of IL-1β from lipopolysaccharide-activated macrophages, adhesion of monocytes to endothelial cells, production of monocyte chemotactic protein-1 and IL-8 in human aortic endothelial cells, LDL-induced proliferation of smooth muscle cells, and activation of NADPH oxidase in human monocytes (for a review, see Brigelius-Flohe et al. 2002a). Most of these processes depend on the activity of PKC, mostly PKCα, and inhibition of PKC by α-tocopherol may well be the common link between these various effects. The described effects of α-tocopherol undoubtedly are anti-inflammatory and should, in consequence, be anti-atherosclerotic and anti-carcinogenic. Such effects, however, have not been observed in clinical trials, which is not easily explained. Most of the effects have been observed in cell cultures only and the culture media usually do not contain α-tocopherol or any other form of vitamin E (Leist et al. 1996). Incubation with α-tocopherol, thus, just restored a normal physiological vitamin E content. This would imply that at a low or deficient vitamin E status the systems in charge of host-defence or acute-phase response, respectively, do not work adequately. In this context it is worth considering that in the clinical trials it was primarily patients or subjects who entered the study with a low vitamin E status that appeared to gain more benefit than those with higher vitamin E plasma levels. Many of the quoted anti-inflammatory actions that are dampened by α-tocopherol involve redox processes or even free-radical reaction and, therefore, may again be attributed to its antioxidant capacity. For a simple antioxidant action, however, a linear dose–response is typical, while a plateau effect near common physiological intakes speaks in favour of a specific interaction of α-tocopherol with defined molecular targets. In practical terms, the saturatable dose–effect relationship predicts that an adequate α-tocopherol status but not a ‘supranutritional’ supplementation might prove to be pivotal to the maintenance of the endogenous defence systems.

Novel functions of vitamin E also comprise the regulation of gene activity. The regulated genes were either individually identified to respond to different vitamers or by global gene expression analyses in experimental animals fed with different forms and concentrations of vitamin E. The first microarray data were obtained from livers of rats fed a vitamin E- and Se-deficient diet. The specific effect of either vitamin E or Se was assessed by analysing genes, the expressing of which could be restored by feeding only one of the two micronutrients (Fischer et al. 2001). Another study used pregnant rats fed α-tocopherol plus a tocotrienol-enriched diet, and gene expression was analysed in the fetal brains (Roy et al. 2002). Two studies investigated the effect of vitamin E deficiency on the male reproductive tract in rats. In the epididymis, mainly genes encoding oxidative stress-related proteins were described to be regulated (Jervis & Robaire, 2004), whereas in testes vitamin E deficiency time-dependently up regulated enzymes involved in testosterone synthesis and cell cycle progression (Rota et al. 2004). In liver, α-tocopherol up regulated γ-glutamylcysteine synthetase, which correlated with an increase of liver glutathione, and down regulated the CD36 scavenger receptor, coagulation factor IX and 5-α-steroid reductase type I also correlating with functional parameters (Barella et al. 2004). The down regulation of CD36 in aortic smooth muscle cells deserves special interest, since it might explain the sometimes observed anti-atherosclerotic effect of α-tocopherol in experimental animals (Ricciarelli et al. 2000). Most interesting findings came from gene expression analyses in the brain of α-TTP-deficient mice. In the cortex of these mice, genes involved in the myelination and synaptogenesis were much less expressed than in wild-type controls (Gohil et al. 2003). A hierarchical cluster analysis further suggested that α-TTP might be required for normal functioning of glial cells and oligodendrocytes (Gohil et al. 2004). A more detailed investigation of α-tocopherol-regulated genes in the brain will finally provide a molecular basis for neurological disorders that develop in vitamin E deficiency (Hayton et al. 2006).

Some of the α-tocopherol-regulated genes almost consistently show up in the lists published in the quoted papers. They were grouped by Azzi et al. (2004) into five clusters – genes that are involved in:

1. the uptake and degradation of vitamin E;
2. lipid uptake and atherosclerosis;
3. the modification of extracellular proteins;
4. inflammatory processes;
5. cellular signalling and cell cycle control.

A systematic analysis of the α-tocopherol response has not yet been seriously attempted. Only Gohil et al. (2003, 2004) tried to find transcription factors that are common to the regulated genes. They found the retinoic acid-related orphan receptor (ROR) α, which belongs to the family of nuclear receptors, repressed in the cortex and adrenal glands of α-TTP-deficient mice. RORα-knockout mice develop the ataxia and memory dysfunction typical for vitamin-E and α-TTP deficiency, which points to RORα as a potential target of α-tocopherol. However, by no means all α-tocopherol-regulated genes are dependent on RORα, and α-tocopherol did not activate an ROR-α-driven reporter gene (R Brigelius-Flohe, unpublished results).

A nuclear receptor specifically binding vitamin E, as is known for vitamin D and A (for a review, see Aranda & Pascual, 2001), has not been identified yet. However, there are some hints that vitamin E is able to activate nuclear receptors in principle. The activation of the PXR-driven
reporter gene in HepG2 cells (Landes et al. 2003) and the up regulation of peroxisome proliferator activated receptor-γ mRNA and activity (De Pascale et al. 2006; Hsieh et al. 2006; Munteanu et al. 2006) points in this direction. A direct binding of tocotrienols but not tocopherols to PXR has been demonstrated (Zhou et al. 2004). However, PXR is not very likely to mediate the biological signals typical for vitamin E. PXR reacts with a large number of structurally highly diverse compounds which are recognised as foreign and, thus, are prone to be eliminated (Watkins et al. 2001). The capability of α-tocopherol and tocotrienols to induce PXR-driven gene expression rather indicates that high concentrations (in the case of α-tocopherol) and the substances as such (in the case of tocotrienols) are destined to be eliminated instead of mediating cellular signals. There remains the possibility that the gene regulation by α-tocopherol is also mediated by PKC inhibition, which could prevent a pivotal phosphorylation of transcription factors, co-activators, or repressors. The increased retinoic acid-induced phosphorylation of RXR and subsequent activation of CRABP-II gene expression upon α-tocopherol treatment of fibroblasts is a first hint supporting this hypothesis (Gimeno et al. 2004).

**β-Tocopherol**

Our diet contains only low amounts of β-tocopherol. Reasonable concentrations can only be found in wheat germ, soyabean or sunflower-seed oil (Stone & Papas, 2003). This may be the reason why studies exploring an isolated biological effect of β-tocopherol are scarce. In most cases β-tocopherol has only been used to demonstrate that α-tocopherol effects were unique and antioxidant-independent. Although β-tocopherol has similar antioxidant properties as α-tocopherol, none of the α-tocopherol-induced effects could be mimicked by β-tocopherol. The effects were either absent or substantially lower with β-tocopherol. For example, activation of PKC, resulting in an inhibition of PKC-dependent activation of NADPH oxidase by phorbol myristate acetate in monocytes (Cachia et al. 1998), was inhibited by α- but not by β-tocopherol (Boschoboinik et al. 1991). Similarly, up regulation of α-tropomyosin (Aratri et al. 1999) and down regulation of CD36 (Ricciarelli et al. 2000) was only triggered by α- and not by β-tocopherol. Also, β-tocopherol did not inhibit the proliferation of smooth muscle cells, whereas γ-, δ- and α-tocopherol were equally inhibitory (Chatelain et al. 1993). The lack of β-tocopherol efficacy in these systems does not comply with its biological activity in the rat gestation–resorption assay, which is 57 % that of α-tocopherol (see earlier), i.e. relatively high. Also, at least in cultured cells, its metabolic rate is almost comparable with that of α-tocopherol (Birringer et al. 2001), i.e. equally low. Thus, the low activity of β-tocopherol, when tested for the novel functions, underscores the specificity displayed by α-tocopherol. However, the efficacy of both tocopherols in the resorption–gestation assay also reveals that the latter depends on a common metabolic pathway that remains to be elucidated.

**γ-Tocopherol**

γ-Tocopherol is the major form of vitamin E in many plant seeds. Due to the high consumption of soyabean products it is also the major form of vitamin E in the US diet (Stone & Papas, 2003). However, as discussed earlier, the plasma concentration in human subjects and experimental animals irrespective of the dosages ingested hardly exceeds 10% of that of α-tocopherol. Due to the lack of one methyl group at the chroman ring, γ-tocopherol is a slightly less powerful antioxidant than α-tocopherol (Kamal-Eldin & Appelqvist, 1996). The presence or absence of the methyl group at position 5 in the chroman structure determines the mode of action of tocopherols with reactive nitrogen oxide species. The reaction of γ-tocopherol with reactive nitrogen oxide species, such as peroxynitrite (ONOO⁻), nitrogen dioxide (NO₂⁻) or nitrogen dioxide-like species, results in the formation of 5-nitro-γ-tocopherol (Christen et al. 1997; Hoglen et al. 1997) (Fig. 2). In contrast, the reaction of α-tocopherol with nitrogen dioxide fundamentally differs. α-Tocopherol, due to the methyl group in position 5, is oxidised to α-tocopheryl quinone, and it cannot be nitrated.

Reactive nitrogen oxide species are generated in inflammatory processes. They have also been associated with inflammation-related diseases such as cancer, CVD and neurodegenerative disorders. Consequently, the capacity of γ-tocopherol to inhibit inflammatory processes has been tested. γ-Tocopherol decreased prostaglandin E₂ production by inhibiting cyclo-oxygenase (COX) 2 activity in cultured cells (Jiang et al. 2000) and in rats (Jiang & Ames, 2003). Anti-inflammatory effects of γ-tocopherol and the putatively underlying mechanism of trapping reactive nitrogen oxide species have been extensively reviewed recently (Jiang et al. 2001; Wagner et al. 2004).

γ-Tocopherol has gained huge interest because of its putative capacity to prevent cancer. Indeed cancer-related processes responded much better to γ-tocopherol than to α-tocopherol. The types of cancers which best respond to vitamin E are prostate and colon cancer. α-Tocopherol supplementation reduced prostate cancer incidence by 32 % in the ‘Alpha-Tocopherol Beta Carotene Cancer Prevention’ study (Heinonen et al. 1998). The association of α-tocopherol, γ-tocopherol and Se with the incidence of prostate cancer was investigated in a nested case–control study (Helzlsouer et al. 2000). Men in the highest quintile of plasma γ-tocopherol levels had a 5-fold lower risk of prostate cancer compared with those in the lowest quintile. Significant protective effects of high levels of α-tocopherol and Se were only observed when γ-tocopherol concentrations were high. In the recently published CLUE studies, a reduced risk of prostate cancer in subjects of the highest quintile of serum γ-tocopherol was reported (Huang et al. 2003). Also a nested control study within the ‘Alpha-Tocopherol Beta Carotene Cancer Prevention’ study cohort revealed that men with higher circulating levels of α-tocopherol and γ-tocopherol had a lower prostate cancer risk (Weinstein et al. 2005). In summary, the studies, which have been reviewed elsewhere (Campbell et al. 2003a; Hensley et al. 2004), reveal stronger evidence for an anticarcinogenic action of γ- than of α-tocopherol.
Also, experimental evidence speaks in favour of a chemopreventive role of \( \gamma \)-rather than of \( \alpha \)-tocopherol. \( \gamma \)-Tocopherol was much more effective in inhibiting proliferation of colon (CaCo2), prostate (LNCaP, DU-145), and osteosarcoma cell lines than \( \alpha \)-tocopherol (Gysin et al. 2002). Similarly, proliferation of PC-3 prostate cancer cells was inhibited by \( \gamma \)-tocopherol (Galli et al. 2004) and finally \( \gamma \)-tocopherol was a more potent inhibitor of neoplastic transformation in 3-methylcholanthrene-treated C3H/10T1/2 murine fibroblasts than \( \alpha \)-tocopherol (Cooney et al. 1993). The underlying mechanisms might be: induction of apoptosis, as has been shown for the LNCaP prostate cancer cells (Jiang et al. 2004); down regulation of cyclins D1 and E1 (Gysin et al. 2002; Galli et al. 2004); suppression of ras-p21 expression, which is often up regulated in colon cancer tissue and is, therefore, taken as an early biomarker for colon cancer (Stone et al. 2002); or up regulation of peroxisome proliferator activated receptor-\( \gamma \) (Campbell et al. 2003b).

However, \( \gamma \)-tocopherol is not only toxic for cancer cells. It inhibited cell viability in mouse macrophages at concentrations above 20 \( \mu \)M, i.e. concentrations similar to those needed for damaging cancer cells. In contrast, human hepatocytes and bovine endothelial cells were not affected (McCormick & Parker, 2004). Hepatocytes but not macrophages metabolised \( \gamma \)-tocopherol, indicating that metabolism might prevent the toxicity of \( \gamma \)-tocopherol. It remains to be investigated whether \( \gamma \)-tocopherol can specifically kill cancer cells and, if so, whether only cancer cells not able to metabolise \( \gamma \)-tocopherol would be killed, leaving healthy cells unaffected.

\( \delta \)-Tocopherol

Although \( \delta \)-tocopherol is present in high amounts, for example, in soyabean, safflower-seed and wheat-germ oil and, thus, at least in the American diet (Stone & Papas, 2003), it has not received much attention and individual functions have not yet been investigated in detail. This might be a consequence of its low biological activity and its low level in plasma and tissues that only reach about 1\% of that of \( \alpha \)-tocopherol (Traber & Kayden, 1989). The discrepancy between the dietary intake and the accumulation in plasma and tissues suggests a high metabolic rate. In fact, the CEHC which was detected \textit{in vivo} first was \( \delta \)-CEHC (Chiku et al. 1984). In cell culture, \( \delta \)-tocopherol is degraded to a substantial amount (Birringer et al. 2001); its \textit{in vivo} route of elimination, however, has not been monitored in detail. In cultured cells, \( \delta \)-tocopherol is the most toxic tocopherol; only 10 \( \mu \)M reduced cell viability of macrophages by more than 90\% (McCormick & Parker, 2004). Toxicity was associated with the induction of apoptosis. As discussed earlier for \( \gamma \)-tocopherol, hepatocytes were not sensitive for \( \delta \)-tocopherol, which again might be explained by their capacity to metabolise and eliminate \( \delta \)-tocopherol. Interestingly, long-term exposure to \( \delta \)-tocopherol led to development of resistance to cytotoxicity. This shows that \( \delta \)-tocopherol also might be able to influence gene expression. The \textit{in vivo} relevance of these observations has to be evaluated.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{tocopherol_reactions.png}
\caption{Reactions of \( \alpha \)- and \( \gamma \)-tocopherol with nitrogen dioxide.}
\end{figure}
studies, however, suggest that tocopherols that are not \( \alpha \)- are eliminated, possibly to prevent cytotoxicity (McCormick & Parker, 2004).

**Tocotrienols**

Tocotrienols are less abundant in the human diet than tocopherols. Only palm oil, rice bran, oat, and barley contain reasonable amounts (Stone & Papas, 2003). Their high antioxidant, anti-cancerogenic properties in vitro and in experimental animals (for a review, see Sen et al. 2000) have recently brought them into focus.

Cell-culture work shows that tocotrienols can inhibit the growth of normal primary mouse mammary epithelial cells (McIntyre et al. 2000b), of pre-neoplastic and even more of neoplastic mouse mammary epithelial cells (McIntyre et al. 2000a). The higher inhibitory potency of tocotrienols, as compared with tocopherols, was attributed to a preferential uptake of tocotrienols by these cells. Also, the proliferation of human breast cancer cell lines was suppressed by tocotrienols (Nesaretam et al. 1998). In all cases \( \gamma \)- and \( \delta \)-tocotrienol exerted the highest inhibitory effects. As underlying mechanisms, inhibition of epidermal growth factor receptor signalling (Sylvester et al. 2002), induction of apoptosis (Takahashi & Loo, 2004) and inhibition of HMG-CoA reductase activity (Mo & Elson, 2004) or increasing HMG-CoA reductase degradation (Theriault et al. 2002) have been discussed. In experimental animals a dietary intake of palm oil rich in tocotrienols suppressed carcinogen-induced mammary tumorigenesis (Sylvester et al. 1986; Nesaretam et al. 1992). The main effect of tocotrienols on CVD parameters is the inhibition of the surface expression of vascular cell adhesion molecule 1 and E-selectin in human umbilical vein endothelial cells and subsequent monocyte adhesion (Theriault et al. 2002).

Whereas the lowering of cholesterol, which has been observed in vitro, was also observed in some experimental animals (for a review, see Schaffer et al. 2005), human studies yielded contradictory results (O’Byrne et al. 2000; Kerckhoff et al. 2002; Qureshi et al. 2002). Only a limited number of clinical trials performed with tocotrienols are available. In a double-blind, randomised, parallel-design study with sixty-seven healthy hypercholesterolaemic subjects who consumed commercially available tocotrienols or placebo for 28 d, no beneficial effects on key CVD risk factors, such as \( \delta \)-iso-prostaglandin F \( 2 \) \( \alpha \) excretion, level of LDL-cholesterol or glucose, have been observed (Mustad et al. 2002).

Tocotrienols are also gaining interest in neuroprotection. Vitamin E deficiency is associated with a progressive neurological syndrome in man, and with increased levels of oxidative damage markers in the brain (for a review, see Berman & Brodaty, 2004). The potential neuroprotective effects of vitamin E have, therefore, been attributed to its antioxidant property. The Alzheimer’s Disease Cooperative Study, an earlier interventional trial with 341 patients, has shown that 2000 IU (900mg) all \( \alpha \)-tocopherol slow down functional deterioration in patients with moderate Alzheimer’s disease (Sano et al. 1997). A recent trial with 769 patients, however, did not detect any benefit of \( \alpha \)-tocopherol supplementation in patients with mild cognitive impairment (Petersen et al. 2005). Thus, a protective effect of even huge amounts of \( \alpha \)-tocopherol in addition to the dietary intake did not ameliorate the disease development. Supplementation with such high amounts of \( \alpha \)-tocopherol so far only ameliorated ataxia in patients with a defect in the gene for \( \alpha \)-TTP. This, however, only underscores that a functional \( \alpha \)-TTP is required for an adequate \( \alpha \)-tocopherol supply to the brain.

In contrast, \( \alpha \)-tocotrienol has been shown to prevent the glutamate-induced death of T4 hippocampal neuronal cells at nanomolar concentrations, whereas at these low concentrations \( \alpha \)-tocopherol did not show any effect (Sen et al. 2000). High extracellular levels of glutamate, as observed in neurological disorders, inhibit the amino acid transport system, \( x_C^- \), which specifically takes up cysteine in exchange for glutamate. Cystine is intracellularly reduced to cysteine, the precursor of glutathione. Thus, inhibition of \( x_C^- \) by glutamate is considered to cause intracellular oxidative stress due to low GSH levels and, in consequence, to induce apoptosis. The molecular basis of the protective effect of tocotrienols was discussed as an induction of extracellular signal-regulated kinase by activation of c-Src or the prevention of 12-LOX activation by intracellular glutathione; thus, interference with the eicosanoid pathway (Sen et al. 2004). Even if the concentrations of tocotrienol needed for the efficacy are very low, it remains to be investigated whether they can be reached in the brain by feeding tocotrienols. Tissue levels of tocotrienols usually are very low (Podda et al. 1996) and also plasma levels cannot substantially be increased by tocotrienol supplementation (Lodge et al. 2001). \( \alpha \)-Tocotrienol in plasma reached maximum \( 1 \mu \text{mol/l} \) in human subjects irrespective of the amount of supplementation (Mustad et al. 2002), levels of \( \gamma \)- and \( \delta \)-tocotrienol being even lower. Furthermore, half-lives of all tocotrienols investigated in human subjects (\( \alpha \), \( \gamma \), \( \delta \)) were 4.5–8.7 times shorter than those of \( \alpha \)-tocopherol (Yap et al. 2001; Schaffer et al. 2005).

A recent animal study supports the concern about the in vivo efficacy of tocotrienols. It had been shown in cell-culture studies that almost all forms of vitamin E can activate a PXR-driven reporter gene and the expression of endogenous CYP3A4 (Landes et al. 2003) (see earlier). By far the highest efficacy was observed with \( \alpha \)- and \( \gamma \)-tocotrienol. Also in vitro binding of tocotrienols to PXR was high, whereas tocopherols were only marginally active in the competitive ligand-binding assay (Zhou et al. 2004). In an attempt to demonstrate the in vivo relevance of the in vitro findings, mice were fed high dosages (250 \( \mu \text{g/d} \) of \( \gamma \)-tocotrienol for 7 d on top of a diet either deficient, adequate or supranutritional with respect to \( \alpha \)-tocopherol. Neither plasma nor liver \( \alpha \)-tocotrienol levels could significantly be elevated. Also CYP3a11 mRNA, the murine homologue to the human CYP3A4, was not changed by \( \gamma \)-tocotrienol, but was induced by \( \alpha \)-tocopherol. The failure of \( \gamma \)-tocotrienol to up regulate CYP3a11 could easily be explained by an enormous excretion of \( \gamma \)-tocotrienol metabolites in the urine (Kluth et al. 2005). Thus, degradation prevented an accumulation of \( \gamma \)-tocotrienol to the extent required for gene activation. This shows that novel functions of tocotrienols observed in vitro must not
uncritically be extrapolated to the in vivo situation. Thorough analyses of the dosages leading to the required plasma or tissue levels have to be performed before animal or even human studies can be started.

Functions of vitamin E metabolites

γ-CEHC has been detected as Loma Linda University factor α (LLU-α), an endogenous natriuretic factor (Wechter et al. 1996). It was isolated from the urine of uremic patients and chemically characterised as the final metabolite of γ-tocopherol. γ-CEHC, at nanomolar concentrations, but not α-CEHC, promotes Na excretion by blocking a K⁺ channel in the thick ascending limb of the kidney’s Henle loop which prevents K⁺ recycling. The resulting inhibition of the Na⁺/2Cl⁻/K⁺ co-transporter will lead to natriuresis (Murray et al. 1997). γ-Tocotrienol could, thus, be considered to be a vitamin functioning as a hormone precursor. However, although γ-CEHC is the final metabolite from both γ-tocopherol and γ-tocotrienol, neither of the vitamers have ever been observed to cause natriuresis and diuresis. Rats fed the huge dose of 10 mg γ-tocotrienol/d for 3 d after depletion of vitamin E stores for 4 weeks excreted large amounts of γ-CEHC but had an increased urine volume and an accelerated Na excretion only if fed a high-salt diet simultaneously (Saito et al. 2003). The results were later confirmed for γ-tocopherol (Uto et al. 2004). At best, therefore, γ-tocopherol- and γ-tocotrienol-derived γ-CEHC might prevent hypertension and CVD caused by high salt intake only (Saito et al. 2003; Tanabe et al. 2004).

Also, other functions of tocopherol metabolites have been described. As expected, α- and γ-CEHC had antioxidant activity comparable with Trolox at micromolar concentrations (Betancor-Fernandez et al. 2002). γ-CEHC, like γ-tocopherol, but not α-CEHC, possesses anti-inflammatory properties. It inhibited prostaglandin E₂ production by COX2 in lipopolysaccharide-stimulated macrophages or IL-1-treated epithelial cells with a 50 % inhibitory concentration (IC₅₀) of 30 μM, whereas the IC₅₀ of γ-tocopherol was about 5–10 μM depending on the cell type investigated (Jiang et al. 2000). α-CEHC was able to inhibit nitrite and prostaglandin E₂ release from TNFα-stimulated EOC-20 murine microglia, indicating inhibition of inducible nitric oxide synthase and COX2 activity and induction respectively (Hensley et al. 2004). The IC₅₀ here also was high (58 μM) and comparable with the IC₅₀ of non-steroidal anti-inflammatory drugs. These huge concentrations of metabolites would never be reached in vivo. Basal plasma concentrations of α-CEHC are about 13 nM (Galli et al. 2002) or 11 nM (Stahl et al. 1999), respectively. Those of γ-CEHC are 161 (Galli et al. 2002) or 66 nM (Stahl et al. 1999). α-CEHC levels could be enhanced to about 200 nmol/l after 7 weeks supplementation with 335 mg RRR-α-tocopherol (Stahl et al. 1999) and γ-CEHC transiently increased up to 30-fold after an application of 100 mg ²H-labelled γ-tocopheryl acetate (Galli et al. 2002). Also a supplementation with γ-tocopherol or γ-tocotrienol at dosages effective for natriuresis in rats (10 mg per rat would mean about 3–4 g per individual which is beyond the tolerable upper intake level) cannot be recommended. Thus, a biological effect of dietary γ-tocopherol or γ-tocotrienol derived γ-CEHC remains to be established.

Is inhibition of metabolism a solution?

Assuming that vitamin E metabolite concentrations required to exert beneficial effects in vitro can never be reached in vivo, prevention of degradation of parent vitamers might be the method of choice to save their biological effects. Whereas this strategy is not needed for α-tocopherol, it might help to increase the plasma and tissue concentration of γ-tocopherol and the tocotrienols.

Evidence for a CYP-catalysed degradation of vitamin E initiated the search for inhibitors of vitamin E metabolism. Among the known CYP-inhibitors tested, ketoconazole exhibited the highest inhibitory effect on γ-tocopherol degradation (Parker et al. 2000). Since ketoconazole is a potent inhibitor of the CYP3A family of P450-isoenzymes, these findings led the authors to conclude that CYP3A4 might be the enzyme responsible for the initial step in vitamin E degradation. The view was revised in a later publication in which preference was given to CYP4F2, which, nevertheless, also was inhibited by ketoconazole (Sontag & Parker, 2002). Whatever form of CYP will finally be identified as the vitamin E-ω-hydroxylase, or whether there are different forms for α- and γ-vitamers, it is generally accepted that CYP are involved.

The involvement of CYP can now explain a number of previous observations: the elevation of plasma and tissue levels of α-tocopherol and especially of γ-tocopherol in rats fed a diet containing sesame seeds or sesame oil (Yamashita et al. 1992, 1995; Kamal-Eldin et al. 2000). Elevation of α-tocopherol in rat brain by feeding sesame seed was even more effective than supplementation with a ten-fold higher dosage of pure α-tocopherol (Abe et al. 2005). Also in human subjects sesame oil increased serum γ-tocopherol concentrations by 10 %, whereas there was no difference between the groups fed canola or rapeseed oil (Kamal-Eldin et al. 2000). α-Tocopherol concentrations were found to be significantly higher in sesame oil-rich diets than in margarines containing high levels of α-tocopherol (Ove et al. 1996). However, whether CYP3A4 is involved in the metabolism of γ-tocopherol remains to be established.

Interestingly, other dietary phenolic compounds also affect concentrations of tocopherols. Curcumin raised the level of α-tocopherol in the lung but not in other tissues or plasma (Kamal-Eldin et al. 2000). Rats fed anthocyanins (Frank et al. 2002), or caffeic acid (Frank et al. 2003a) had higher γ-tocopherol contents in some tissues, and feeding of catechin enhanced α-tocopherol in rats but did not inhibit δ-tocopherol hydroxylation in HepG2 cells (Frank et al. 2003b). Whether catechin indeed acts via an ω-hydroxylase-independent mechanism as suggested, or whether α- and δ-tocopherol are degraded by different
enzymes and, therefore, respond differently to catechins, remains to be elucidated. Thus, it might be possible to elevate the levels of individual forms of vitamin E in plasma or in certain tissues by a specific inhibition of the enzymes which are responsible for their elimination. It might indeed be the better strategy to enhance the levels of vitamin E by a dietary regimen, for example, by increasing the intake of vegetable oils containing lignans such as sesamin instead of taking high dosages of supplements with unknown side effects. Whether this is sufficient to give, for example, γ-tocopherol the chance to exert its novel functions that have been elucidated over the last few years remains to be investigated.

Conclusions
The name-giving biological potential of tocopherol, i.e. to promote birth, is not yet understood at the molecular level. Instead, a variety of cellular events, mostly specifically exerted by RRR-α-tocopherol, are showing up at the horizon. These newly discovered effects of individual vitamers comprise modulation of signalling cascades and gene regulation, but have so far been demonstrated in tissue culture, exceptionally in experimental animals, and accordingly await proof of relevance to human health.

Most clinical trials with vitamin E have been based on the misconception of tocopherols and tocotrienols simply acting as lipophilic antioxidants. Accordingly, the dosages chosen were usually supranutritional, little attention was paid to the form of vitamin E since the antioxidant potential is similar, and the trial endpoints were prevention or delayed progression of diseases believed to be caused or aggravated by oxidative stress, such as CVD, cancer or neurodegenerative symptoms. Little, if any, benefit of vitamin E could convincingly be demonstrated, although the trials had enrolled tens of thousands of patients. In fact, the only clinical condition that reliably responded to mega-doses of vitamin E is a genetic defect in α-TTP, which, untreated, results in a generalised vitamin E deficiency with a characteristic neurological syndrome. In retrospect, the antioxidant concept of vitamin E supplementation had to fail, since none of the vitamers or their metabolites ever reaches tissue levels that could efficiently counteract an unspecific oxidative stress.

Emerging knowledge now reveals that each individual vitamer has a pharmacodynamic profile of its own. The mammalian organism shows a particular preference for RRR-α-tocopherol in terms of specific binding, distribution and retention by means of specialised proteins such as α-TTP. The other vitamers are degraded and eliminated fast like ‘unwanted’ xenobiotics. Under this consideration it appears questionable if their particular pharmacodynamic profile, as it is observed in tissue culture, can ever be exploited in vivo. As far as the ‘real’ vitamin RRR-α-tocopherol is concerned, the evidence for prevention or cure of diseases (apart from α-TTP deficiency) by supranutritional dosages is equally lacking.

In view of past disappointments, it appears advisable to head for a detailed understanding of how the individual vitamers work at the molecular level and how they are handled under healthy and diseased conditions by the organism, before embarking on new clinical mega-trials that at present cannot be based on a solid scientific concept.

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