REVIEW

Regulation of xenobiotic metabolism, the only signaling function of α-tocopherol?

Maret G. Traber

Linus Pauling Institute, Oregon State University, Corvallis, OR, USA

There is growing concern based on meta-analyses of clinical trials using vitamin E supplements that these supplements increase the risk of all-cause mortality in humans. My laboratory has been investigating the metabolism and disposition of ''excess'' vitamin E. This review focuses on the various mechanisms that prevent vitamin E intoxication. Non-α-tocopherols are aggressively metabolized thereby preventing their tissue accumulation and limiting increases in their plasma concentrations. Moreover, ''excess'' α-tocopherol is also metabolized and its concentrations are limited. The mechanisms for this limitation do not seem to be specific for vitamin E, but rather are general xenobiotic pathways. We suggest that the most relevant cytochrome P450-mediated pathway is the one that is most important for the regulation and activation of vitamin K, specifically the one dependent on CYP4F2.

Received: September 8, 2009
Revised: October 8, 2009
Accepted: October 19, 2009

Keywords:
α-CEHC / α-Tocopherol / Cytochrome P450 / Mortality / Vitamin K

1 Introduction

For decades, vitamin E has been considered to be a ''safe'' vitamin [1]. Indeed, the Food and Nutrition Board [2] in 2000 used data from rat toxicity studies to estimate an upper tolerable limit for humans taking vitamin E supplements because insufficient data were available from human studies. An estimated ''uncertainty factor'' of 36 was used to reduce the adverse level observed in the rats, equivalent to 35 000 mg human (70 kg) consumption, to the upper tolerable limit value of 1000 mg. In light of these data, meta-analyses of the outcomes of vitamin E supplementation trials were, therefore, surprising. These meta-analyses claimed that 400 IU (~400 mg) vitamin E supplements could increase all-cause mortality [3, 4]; certainly, a worse outcome than bleeding episodes observed in rats [5, 6] or in humans [7]. Subsequently, others using the same clinical data as the meta-analyses, but other statistical approaches, claimed ''Vitamin E intake is unlikely to affect mortality regardless of dose'' [8]. To add to the confusion, the latest epidemiologic study suggests that vitamin E supplements decrease all-cause mortality, and specifically cardiovascular disease mortality [9].

Seemingly, the solution to the confusion would be to document the mechanism for adverse vitamin E effects. The problem is that there is little consistency between clinical trials as to what are adverse effects of excess vitamin E. Increased left ventricular dysfunction was observed in vitamin E takers in the HOPE TWO trial in cardiovascular disease patients [10], but was not observed in two vitamin E intervention trials of normal healthy subjects [11, 12]. Sesso *et al.* [12] reported ''an increased risk of hemorrhagic stroke (HR, 1.74 [12]; $P = .04$)'' in male vitamin E takers, while they did not find differences between groups with respect to tendency to bleed, although the bleeding tendency may have been similar in the two groups because nearly 80% of the subjects regularly took aspirin. By contrast, Lee *et al.* [11] observed an increase in nosebleeds (epistaxis) in women taking vitamin E in the Women's Health Study. Moreover, Glynn *et al.* [13] reported in women from the same study that vitamin E reduced the risk of thromboembolism, while aspirin did not. The authors [13] suggested that vitamin E's anticoagulant effect could result from interference with vitamin K, although a mechanism was not

Correspondence: Professor Maret G. Traber, Linus Pauling Institute 571, Weniger Hall Oregon State University, Corvallis, OR 97331, USA
E-mail: Maret.Traber@oregonstate.edu
Fax: +1-541-737-5077

Abbreviations: CEHC, carboxyethyl hydroxychroman; **CYP,** cytochrome P450; **PXR,** pregnane X receptor

suggested. We [14] have suggested that vitamin E may interfere with vitamin K activation or metabolism because a portion, *e.g.* the phytyl tail, of the vitamin E and vitamin K structures are similar.

Unlike other fat-soluble vitamins, vitamin E is not accumulated in the liver to toxic levels, suggesting that excretion and metabolism are important in preventing adverse vitamin E effects, supporting the conclusion that it is unlikely that vitamin E supplements increase risk of mortality. This brings us to the purpose of this review, that is, the discussion of vitamin E metabolism as a mechanism to prevent the over-accumulation of vitamin E, as well as the role of vitamin E in altering xenobiotic metabolism. Finally, the interaction of vitamin E with relationship to vitamin K activation will be discussed.

2 Vitamin E metabolism as a mechanism to prevent the accumulation of non-α-tocopherol forms

Differences in the biologic activities of the eight different naturally occurring forms of vitamin E were first recognized over 80 years ago [15]. Although differences in antioxidant activity might have explained those differences, they did not, as reviewed [16]. Specifically, the antioxidant activities of natural and synthetic α-tocopherols are identical, but they have different biologic activities; these differences were first described over 50 years ago [17]. Progress in defining the mechanisms for differences in biologic activity came with the discovery of the hepatic α-tocopherol transfer protein [18, 19] and its cloning [20, 21]. Then, vitamin E deficient humans were described who had a genetic defect in the α-tocopherol transfer protein [22, 23]. These people not only developed vitamin E deficiency symptoms, but they also were unable to discriminate between various forms of vitamin E [24, 25], suggesting that the α-tocopherol transfer protein was critical for discrimination between vitamin E forms. Thus, the α-tocopherol transfer protein seemed to be the mechanism for the observed preference for natural α-tocopherol; however, that finding was not the whole explanation.

Now it is clear that vitamin E metabolism is a key mechanism for the preference for α-tocopherol, for limiting α-tocopherol accumulation and for determining the circulating levels of various vitamin E forms. The vitamin E metabolite derived from α-tocopherol and from α-tocotrienol (α-CEHC (carboxyethyl hydroxychroman) is tail-shortened, but has an unoxidized head group (Fig. 1) [26]. Nearly 60 years ago, Simon *et al.* [27] described the steps and end products of vitamin E metabolism, which were called ''Simon metabolites.'' The products described are both CEHC and its oxidized product [26]. Modern techniques to prevent *in vitro* oxidation have shown that Simon metabolites largely occur as a result of poor sample handling and *in vitro* oxidation [28]. Thus, the biologically relevant vitamin E metabolites are CEHCs.

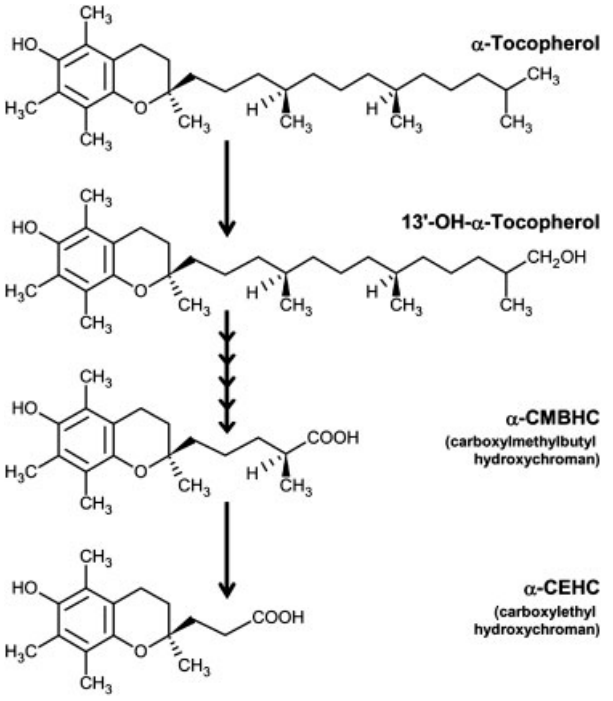

Figure 1. Scheme for α-tocopherol metabolism.

The major route of γ-tocopherol excretion was proposed to be *via* its metabolic product, γ-CEHC [29]. In cultured hepatocytes, γ-CEHC production is 100 times greater than is α-CEHC from similar amounts of tocopherols added to the medium [30]. Studies using deuterium-labeled α- and γ-tocopherols demonstrated that the preference for α-tocopherol depended upon the rapid metabolism of γ-tocopherol to γ-CEHC [31]. This conclusion is emphasized by studies in Drosophila, which documented that the flies lack the α-tocopherol transfer protein, but were capable of metabolizing both δ- and γ-tocopherols to their respective CEHCs. Importantly, the flies preferentially retained α-tocopherol. Thus, the rate of α-tocopherol's metabolism is relatively low compared with the rates of other vitamin E forms, thereby explaining the α-tocopherol accumulation.

Vitamin E is absorbed from the diet and is delivered to liver, which is responsible for secreting α-tocopherol into the plasma *via* the α-tocopherol transfer protein mechanism, as reviewed [32]. Apparently, the liver plays a major role in vitamin E metabolism. In rats injected subcutaneously with α-tocopherol, despite massive increases in liver α-tocopherol, other tissues only increased two- to threefold α-tocopherol, and substantial α-CEHC increases were observed in the liver [33]. Plasma vitamin E regulation was also observed in humans taking a mixed tocopherol and tocotrienol preparation (623 mg γ-tocopherol, 61.1 mg α-tocopherol, 11.1 mg β-tocopherol and 231 mg σ-tocopherol (tocotrienols) *per* capsule), followed by a 1-wk washout period, and then a second dosing period in which volunteers consumed two capsules/day for 1 wk [34]. In this study although circulating γ-tocopherol concentrations increased fourfold by the end of

the first week, α-tocopherol concentrations were unchanged. By contrast, γ-CEHC increased tenfold, and at the end of the second dosing period γ-tocopherol did not increase further, while γ-CEHC increased 20-fold compared to baseline concentrations. Importantly, γ-CEHC concentrations decreased to baseline within 1 wk of supplement cessation. Although the supplement contained tocotrienols, no measures of serum tocotrienols were reported, so γ-CEHC concentrations may also reflect tocotrienol metabolism. In rats, accumulation of α-tocotrienol in various organs could be demonstrated when α-tocotrienol was administered for more than 2 years; however, α-tocotrienol depletion took less than 2 months when the diet was replaced with a vitamin E deficient diet one [35]. Remarkably, the loss of α-tocopherol was negligible [35]. Based on pharmacokinetic studies in humans, it is likely that metabolism of the tocotrienols led to their rapid disappearance [36]. Abe *et al.* [37] elegantly demonstrated the importance of metabolism in determining vitamin E biologic activity. Vitamin E-deficient rats were given by gavage an emulsion containing a mixture of tocopherols and tocotrienols. Plasma α-tocopherol increased, while the other vitamin E forms did not; when ketoconazole, a potent inhibitor of cytochrome P450-dependent vitamin E metabolism in cultured cells, was also given with the vitamin E emulsion; then the metabolism of the other forms was inhibited and they also increased in the plasma. These data also emphasize the importance of cytochrome P450s in vitamin E metabolism.

3 Mechanism of metabolism

Vitamin Es are metabolized by cytochrome P450s (CYPs), conjugated, and excreted in urine [26] or bile [38]. Following ω-hydroxylation by CYPs, ß-oxidation is proposed to take place [27].

In addition to α-CEHC, 13′OH-α-tocopherol and α-CMBHC (carboxymethyl butyl hydroxychroman) have been reported in liver of rats injected with α-tocopherol [39]. Metabolism of other non-α-tocopherol metabolites should generate similar intermediates. CEHCs conjugated with sulfate or glucuronide have been described [29, 40, 41]. Various transporters are likely candidates for mediating hepatic CEHC excretion because CEHCs are found in plasma, urine and bile. Each of these steps in vitamin E metabolism is discussed below.

3.1 Cytochrome P450-mediated vitamin E metabolism

CYP3A has been proposed to be involved in vitamin E metabolism based on the observation that CYP3A inhibitors and stimulators altered CEHC production [30, 42–44]. Additionally, studies in mice demonstrated that feeding α-tocopherol increased Cyp3a mRNA [45]. (Note that CYP protein nomenclature is capitalized, while only the C is capitalized in mice). However, analysis of individual human cytochrome P450s expressed in insect cells led to a different conclusion; these latter studies identified CYP4F2 to be the CYP involved in the ω-oxidation of γ-tocopherol [46]. Subsequent studies by Sontag and Parker [47] defined that CYP4F2 activity toward α-tocopherol was limited relative to other forms of vitamin E, but importantly that α-tocopherol stimulated the ω-hydroxylation of non-α-tocopherol forms of vitamin E. Thus, they termed CYP4F2 the tocopherol ω-hydrolase [46].

The studies by Sontag and Parker [46, 47] would seemingly define CYP4F2 as the P450 involved in vitamin E metabolism. However, we [48] found that within 3 days of daily vitamin E injections to rats, both CYP3A and CYP2B protein nearly doubled and remained elevated through day 18; neither CYP4F nor the α-tocopherol transfer protein concentrations changed. These data suggest that α-tocopherol does not regulate CYP4F. However, inhibitors of CYP4F, such as an omega-imidazole containing compound, 1, [(R)-2-(9-(1H-imidazol-1-yl)nonyl)-2,5,7,8-tetramethylchroman-6-ol] do decrease CEHC production from γ-tocopherol in HepG2 cells in culture [49]. Thus, *in vivo* CYP4F2 most likely initiates vitamin E metabolism, but it is not specific for vitamin E alone.

CYP4 family members, which are expressed in humans, rats and mice, are the major fatty acid ω-hydroxylases, as reviewed [50]. Regulation of CYP4 is not well understood. CYP4A and CYP4F genes are regulated in the opposite direction by peroxisome proliferators, by starvation and by high fat diets [51]. Additionally, it is now appreciated that the CYP4 family modulates eicosanoids during inflammation and metabolizes some clinically significant pharmaceutical agents [52].

CYP4F2 is known for its role in metabolism of eicosanoids [52, 53]. Over-expression of human CYP4F2 in mice led to increased production of 20-hydroxyeicosatetraenoic acid (20-HETE) and increased arterial blood pressure [54]. *In vitro*, sesamin inhibited human renal and liver microsome 20-HETE synthesis and was selective toward CYP4F2 (IC50: 1.9 μmol/L), had activity toward CYP4A11 (IC50: > 150 μmol/L), as well as cytochrome P epoxygenation of arachidonic acid (IC50: > 50 μmol/L) [55]. Moreover, Wu *et al.* [55] found that supplementation of overweight humans with sesame (25 g) reduced the plasma and urinary levels of 20-HETE, but had no significant effect on blood pressure.

Additionally, CYP4F2 variants in the human population have been found to have an altered response to warfarin [56]. Therefore, it is not surprising that insect cells expressing CYP4F2 are able to ω-hydroxylate vitamin K1 (phylloquinone) [57]. This intriguing aspect of vitamin K metabolism will be discussed further below.

3.2 Conjugation controversy

Most investigators have assumed that both sulfate and glucuronide conjugates of CEHC are present in their

samples and have hydrolyzed these to obtain free CEHCs for analysis. Kiyose *et al.* [38] methylated the CEHCs for analysis, but did not address the question of the amount or the form of CEHC conjugates that were initially present in the samples. In contrast, Pope *et al.* [41] synthesized both α-CEHC sulfate and α-CEHC glucuronide and developed a method for their identification using electrospray detection and LC-MS. They reported in α-tocopherol supplemented humans that the urinary α-CEHC glucuronide was present, but the sulfate conjugate could not be unambiguously characterized. In contrast, the Jiang group [58–60] has identified that sulfated-γ-CEHC is the major CEHC conjugate both in rats and in human cells in culture. Sulfated intermediates were found between 13′-OH-γ-tocopherol and γ-CEHC, suggesting that sulfation may be an important early step in intracellular trafficking to guide vitamin E metabolism. It is not clear whether the differences in the two outcomes, either sulfation or glucuronidation, relates to differences in α- and γ-tocopherol metabolism, or differences between rats and humans, or differences of measures from liver compared with urine samples.

3.3 Export from the liver

It appears that one of the hepatic responses to ''excess'' α-tocopherol is to up-regulate α-tocopherol biliary secretion. The multi-drug resistance gene MDR1 (p-glycoprotein, or ABCB1) is modulated by the same xenobiotics that alter CYP3A [61]. Our mouse microarray data [62] indicate that the mouse mdr1 gene is modulated by excess α-tocopherol and the rat MDR protein is up-regulated in response to increasing tissue α-tocopherol concentrations [39]. Previously, mouse mdr2, another ABC transport protein, was shown to be involved in the efflux of α-tocopherol into bile [63].

Thus far there are no reports of transporters specifically involved in the secretion of CEHCs or their conjugates. Hepatic transporters are possible candidates for this process, but only the Slc22a5 gene (Solute carrier family 22, organic anion transporter, member 5) was up-regulated in mice fed high vitamin E diets [62]. Clearly, more research is needed to define the mechanisms for export of vitamin E forms and their metabolites from the liver.

4 Regulation by PXR, or not

Nuclear receptors comprise a superfamily of ligand-activated transcription factors. They can be grouped into (i) receptors for endocrine hormones and (ii) ''orphan'' nuclear receptors, including receptors for fatty acids (peroxisome proliferator activated receptors), bile acids (farnesoid X receptor), oxysterols (liver X receptors), as well as lipophilic xenobiotics (pregnane X receptor (PXR) and constitutive androstane receptor) [64]. These latter two are linked because constitutive androstane receptor and PXR have overlapping functions [65]. However, only PXR has been demonstrated to bind vitamin E [66]. In HepG2 cells transfected with human PXR and the chloramphenicol acetyl transferase gene linked to two PXR responsive elements, chloramphenicol acetyl transferase activity was most strongly induced by α- and γ-tocotrienol followed by rifampicin, δ-, α- and γ-tocopherol [66]. Thus, PXR could be important in vitamin E metabolism and its regulation of xenobiotic pathways.

PXR regulates a constellation of genes involved in xenobiotic detoxification [67–69], including CYP3A [67]. On ligand binding, PXR, binds to its response element in promoter region of genes and induces some cytochrome P450 oxidation systems (phase I), conjugation systems (phase II) and transporters (phase III) [70]. All of these systems are potential mediators of hepatic concentrations of vitamin E and its metabolites. However, Cho *et al.* [71] showed that stimulation of PXR by the mouse PXR activator pregnenolone16a-carbonitrile (PCN) in wild-type compared with PXR-null mice decreased vitamin E metabolism! Moreover, they identified a new CEHC conjugate, a glucoside, using metabolomics techniques and mass spectrometry. Importantly, PCN treatment, which stimulates PXR, decreased the urinary excretion of α-CEHC glucuronide and γ-CEHC glycoside in wild-type but not PXR-null mice.

The importance of PXR in regulating CYP3A has led to the concern that vitamin E might dysregulate pharmaceutical drug metabolism [72, 73]. Nonetheless, vitamin E supplements did not appear to alter efficacy of either simvastatin or lovastatin with regard to hypercholesterolemic patients' reduced cholesterol concentrations [74]. These two drugs were chosen for study because they are both metabolized *via* a CYP3A-dependent mechanism. Werba *et al.* [75] approached this problem by studying the effect of simvastatin (20 mg/day) or pravastatin (40 mg/day) on α- and γ-tocopherol concentrations in hypercholesterolemic humans. Although both drugs decreased circulating cholesterol and α-tocopherol concentrations, only simvastatin raised γ-tocopherol concentrations. These findings suggest that simvastatin and γ-tocopherol competed for the same sites for metabolism, while pravastatin, which is not dependent on CYP3A for metabolism, did not. Taken together with the findings in mice, it appears that stimulation of PXR- or CYP3A-dependent metabolism tends to decrease, rather than increase, vitamin E metabolism. These outcomes are contrary to the expectation that vitamin E functioning as a PXR ligand would increase PXR-responsive events.

5 Vitamin K activation and vitamin E metabolism

Findings from the Women's Health Study showed that vitamin E supplementation significantly decreased venous thromboembolism by 21% [13]. This beneficial vitamin E

effect may be due to interactions between vitamin E and vitamin K metabolism. For example, both vitamins bind to PXR [66, 76, 77]. Although this nuclear receptor regulates xenobiotic metabolism in the liver, in osteoblasts where vitamin K is a PXR ligand, it regulates extracellular matrix and collagen formation [78, 79]. Not only do both vitamin E and K bind to the same nuclear receptor, but they appear to share the same metabolic fates. That is, the side chain of both vitamin E and K are ω-hydroxylated, then ß-oxidized [80].

Vitamin K activation may also be dependent upon this pathway for side-chain metabolism. Plant-derived, dietary vitamin K is phylloquinone (vitamin K1), which has a 20-carbon phytyl side chain, while menaquinones (MK), have multiple prenyl units, as indicated by their suffix number (*i.e.* MK-n) [80]. The liver is thought to convert phylloquinone to menadione, which is then used by extrahepatic tissues for MK-4 synthesis [81]. Importantly, it appears that vitamin E interferes with this process because extrahepatic tissue vitamin K concentrations were lower in rats fed a high vitamin E diet [82]. Additionally, high-dose vitamin E supplementation (1000 IU) in humans increased the degree of under-γ-carboxylation of prothrombin (proteins induced by vitamin K absence-factor II) [83]. Potentially, high-dose vitamin E supplements increase liver α-tocopherol concentrations to the point that α-tocopherol competes with phylloquinone preventing the latter's hepatic transformation to menadione, and thus limiting MK-4 synthesis. Although vitamin E may limit the active form of vitamin K, this vitamin E action may be beneficial. Indeed, Glynn *et al.* [13] proposed that vitamin E supplements would be a safer alternative to warfarin in humans with increased risk of thrombosis.

6 Concluding remarks

Given the potential interaction between vitamins E and K, the lack of specificity of CYP4F2 for vitamin E metabolism, and the relative decrease in vitamin E metabolism upon PXR stimulation by known PXR activators *in vivo*, it appears that vitamin E metabolism occurs non-specifically. That is to say, the increase in vitamin E metabolism observed with increased liver α-tocopherol [39, 45, 62, 84] likely occurs as a result of non-specific mechanisms to up-regulate xenobiotic pathways, rather than a specific α-tocopherol directed mechanism. Thus, with regard to the non-antioxidant mechanisms for vitamin E activity, as we learn more about vitamin E metabolism, it is becoming clearer that vitamin E does not appear to specifically regulate any signaling pathways. This leaves open the question as to whether certain individuals are less capable of preventing vitamin E over-accumulation. Given that the "adverse effect" of anti-coagulation by vitamin E may be beneficial in some individuals, the adverse effects of vitamin E supplements need further study.

The author has declared no conflict of interest.

7 References

[1] Hathcock, J. N., Azzi, A., Blumberg, J., Bray, T. *et al.*, Vitamins E and C are safe across a broad range of intakes. *Am. J. Clin. Nutr.* 2005, *81*, 736–745.

[2] Food and Nutrition Board, Institute of Medicine, *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*, National Academy Press, Washington 2000.

[3] Miller3rd, E. R., Paston-Barriuso, R., Dalal, D., Riemersma, R. A. *et al.*, Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann. Intern. Med.* 2005, *142*, 37–46.

[4] Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., Gluud, C., Mortality in Randomized Trials of Antioxidant Supplements for Primary and Secondary Prevention: Systematic Review and Meta-analysis. *J. Am. Med. Assoc.* 2007, *297*, 842–857.

[5] Takahashi, O., Ichikawa, H., Sasaki, M., Hemorrhagic toxicity of d-alpha-tocopherol in the rat. *Toxicology* 1990, *63*, 157–165.

[6] Takahashi, O., Haemorrhagic toxicity of a large dose of alpha-, beta-, gamma- and delta-tocopherols, ubiquinone, beta-carotene, retinol acetate and L-ascorbic acid in the rat. *Food Chem. Toxicol.* 1995, *33*, 121–128.

[7] Helson, L., The effect of intravenous vitamin E and menadiol sodium diphosphate on vitamin K dependent clotting factors. *Thromb. Res.* 1984, *35*, 11–18.

[8] Berry, D., Wathen, J. K., Newell, M., Bayesian model averaging in meta-analysis: vitamin E supplementation and mortality. *Clin. Trials* 2009, *6*, 28–41.

[9] Pocobelli, G., Peters, U., Kristal, A. R., White, E., Use of supplements of multivitamins, vitamin C, and vitamin E in relation to mortality. *Am. J. Epidemiol.* 2009, *170*, 472–483.

[10] Lonn, E., Bosch, J., Yusuf, S., Sheridan, P. *et al.*, Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. *J. Am. Med. Assoc.* 2005, *293*, 1338–1347.

[11] Lee, I. M., Cook, N. R., Gaziano, J. M., Gordon, D. *et al.*, Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. *J. Am. Med. Assoc.* 2005, *294*, 56–65.

[12] Sesso, H. D., Buring, J. E., Christen, W. G., Kurth, T. *et al.*, Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. *J. Am. Med. Assoc.* 2008, *300*, 2123–2133.

[13] Glynn, R. J., Ridker, P. M., Goldhaber, S. Z., Zee, R. Y., Buring, J. E., Effects of random allocation to vitamin E supplementation on the occurrence of venous thromboembolism: report from the Women's Health Study. *Circulation* 2007, *116*, 1497–1503.

[14] Traber, M. G., Frei, B., Beckman, J. S., Vitamin E revisited: do new data validate benefits for chronic disease prevention? *Curr. Opin. Lipidol.* 2008, *19*, 30–38.

[15] Emerson, O. H., Emerson, G. A., Mohammad, A., Evans, H. M., The chemistry of vitamin E tocopherols from natural sources. *J. Biol. Chem.* 1937, *22*, 99–107.

[16] Traber, M. G., Atkinson, J., Vitamin E, antioxidant and nothing more. *Free Radic. Biol. Med.* 2007, *43*, 4–15.

[17] Harris, P. L., Ludwig, M. I., Relative vitamin E potency of natural and of synthetic α-tocopherol. *J. Biol. Chem.* 1949, *179*, 1111–1115.

[18] Sato, Y., Hagiwara, K., Arai, H., Inoue, K., Purification and characterization of the alpha-tocopherol transfer protein from rat liver. *FEBS Lett.* 1991, *288*, 41–45.

[19] Sato, Y., Arai, H., Miyata, A., Tokita, S. *et al.*, Primary structure of alpha-tocopherol transfer protein from rat liver Homology with cellular retinaldehyde-binding protein. *J. Biol. Chem.* 1993, *268*, 17705–17710.

[20] Min, K. C., Kovall, R. A., Hendrickson, W. A., Crystal structure of human α-tocopherol transfer protein bound to its ligand: Implications for ataxia with vitamin E deficiency. *Proc. Natl. Acad. Sci. USA* 2003, *100*, 14713–14718.

[21] Meier, R., Tomizaki, T., Schulze-Briese, C., Baumann, U., Stocker, A., The molecular basis of vitamin E retention: structure of human alpha-tocopherol transfer protein. *J. Mol. Biol.* 2003, *331*, 725–734.

[22] Ben Hamida, M B. S., Sirugo, G., Ben Hamida, C., Panayides, K. *et al.*, Friedreich's ataxia phenotype not linked to chromosome 9 and associated with selective autosomal recessive vitamin E deficiency in two inbred Tunisian families. *Neurology* 1993, *43*, 2179–2183.

[23] Doerflinger, N., Linder, C., Ouahchi, K., Gyapay, G. *et al.*, Ataxia with vitamin E deficiency: refinement of genetic localization and analysis of linkage disequilibrium by using new markers in 14 families. *Am. J. Hum. Genet.* 1995, *56*, 1116–1124.

[24] Traber, M. G., Sokol, R. J., Burton, G. W., Ingold, K. U. *et al.*, Impaired ability of patients with familial isolated vitamin E deficiency to incorporate alpha-tocopherol into lipoproteins secreted by the liver. *J. Clin. Invest.* 1990, *85*, 397–407.

[25] Traber, M. G., Sokol, R. J., Kohlschütter, A., Yokota, T. *et al.*, Impaired discrimination between stereoisomers of α-tocopherol in patients with familial isolated vitamin E deficiency. *J. Lipid Res.* 1993, *34*, 201–210.

[26] Brigelius-Flohé, R., Traber, M. G., Vitamin E: function and metabolism. *FASEB J.* 1999, *13*, 1145–1155.

[27] Simon, E. J., Eisengart, A., Sundheim, L., Milhorat, A. T., The metabolism of vitamin E II Purification and characterization of urinary metabolites of alpha-tocopherol. *J. Biol. Chem.* 1956, *221*, 807–817.

[28] Schultz, M., Leist, M., Petrzika, M., Gassmann, B., Brigelius-Flohé, R., Novel urinary metabolite of alpha-tocopherol, 2,5,7,8-tetramethyl-2(2′-carboxyethyl)-6-hydroxychroman, as an indicator of an adequate vitamin E supply? *Am. J. Clin. Nutr.* 1995, *62*, 1527S-1534S.

[29] Swanson, J. E., Ben, R. N., Burton, G. W., Parker, R. S., Urinary excretion of 2,7, 8-trimethyl-2-(beta-carboxyethyl)-6-hydroxychroman is a major route of elimination of gamma-tocopherol in humans. *J. Lipid Res.* 1999, *40*, 665–671.

[30] Birringer, M., Pfluger, P., Kluth, D., Landes, N., Brigelius-Flohe, R., Identities and differences in the metabolism of tocotrienols and tocopherols in HepG2 cells. *J. Nutr.* 2002, *132*, 3113–3118.

[31] Leonard, S. W., Paterson, E., Atkinson, J. K., Ramakrishnan, R. *et al.*, Studies in humans using deuterium-labeled α- and γ-tocopherol demonstrate faster plasma γ-tocopherol disappearance and greater γ-metabolite production. *Free Radic. Biol. Med.* 2005, *38*, 857–866.

[32] Traber, M. G., in: Shils, M. E., Olson, J. A., Shike, M., Ross, A. C. (Eds.), *Modern Nutrition in Health and Disease*, Lippincott Williams & Wilkins, Baltimore 2005, pp. 396–411.

[33] Mustacich, D. J., Vo, A. T., Elias, V. D., Payne, K. *et al.*, Regulatory mechanisms to control tissue alpha-tocopherol. *Free Radic. Biol. Med.* 2007, *43*, 610–618.

[34] Wiser, J., Alexis, N. E., Jiang, Q., Wu, W. *et al.*, In vivo gamma-tocopherol supplementation decreases systemic oxidative stress and cytokine responses of human monocytes in normal and asthmatic subjects. *Free Radic. Biol. Med.* 2008, *45*, 40–49.

[35] Patel, V., Khanna, S., Roy, S., Ezziddin, O., Sen, C. K., Natural vitamin E alpha-tocotrienol: retention in vital organs in response to long-term oral supplementation and withdrawal. *Free Radic. Res.* 2006, *40*, 763–771.

[36] Yap, S. P., Yuen, K. H., Wong, J. W., Pharmacokinetics and bioavailability of alpha-, gamma- and delta-tocotrienols under different food status. *J. Pharm. Pharmacol.* 2001, *53*, 67–71.

[37] Abe, C., Uchida, T., Ohta, M., Ichikawa, T. *et al.*, Cytochrome P450-dependent metabolism of vitamin E isoforms is a critical determinant of their tissue concentrations in rats. *Lipids* 2007, *42*, 637–645.

[38] Kiyose, C., Saito, H., Kaneko, K., Hamamura, K. *et al.*, Alpha-tocopherol affects the urinary and biliary excretion of 2,7,8-trimethyl-2 (2′-carboxyethyl)-6-hydroxychroman, gamma-tocopherol metabolite, in rats. *Lipids* 2001, *36*, 467–472.

[39] Mustacich, D. J., Leonard, S. W., Devereaux, M. W., Sokol, R. J., Traber, M. G., α-Tocopherol regulation of hepatic cytochrome P450s and ABC transporters. *Free Radic. Biol. Med.* 2006, *41*, 1069–1078.

[40] Stahl, W., Graf, P., Brigelius-Flohe, R., Wechter, W., Sies, H., Quantification of the alpha- and gamma-tocopherol metabolites 2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman and 2,7,8-trimethyl-2-(2′-carboxyethyl)-6-hydroxychroman in human serum. *Anal. Biochem.* 1999, *275*, 254–259.

[41] Pope, S. A., Burtin, G. E., Clayton, P. T., Madge, D. J., Muller, D. P., Synthesis and analysis of conjugates of the major vitamin E metabolite, alpha-CEHC. *Free Radic. Biol. Med.* 2002, *33*, 807–817.

[42] Birringer, M., Drogan, D., Brigelius-Flohe, R., Tocopherols are metabolized in HepG2 cells by side chain omega-oxidation and consecutive beta-oxidation. *Free Radic. Biol. Med.* 2001, *31*, 226–232.

[43] Parker, R. S., Sontag, T. J., Swanson, J. E., Cytochrome P4503A-dependent metabolism of tocopherols and inhibition by sesamin. *Biochem. Biophys. Res. Commun.* 2000, *277*, 531–534.

[44] Ikeda, S., Tohyama, T., Yamashita, K., Dietary sesame seed and its lignans inhibit 2,7,8-trimethyl-2(2′-carboxyethyl)-6-hydroxychroman excretion into urine of rats fed gamma-tocopherol. *J. Nutr.* 2002, *132*, 961–966.

[45] Kluth, D., Landes, N., Pfluger, P., Muller-Schmehl, K. *et al.*, Modulation of Cyp3a11 mRNA expression by alpha-tocopherol but not gamma-tocotrienol in mice. *Free Radic. Biol. Med.* 2005, *38*, 507–514.

[46] Sontag, T. J., Parker, R. S., Cytochrome P450 omega-hydroxylase pathway of tocopherol catabolism: Novel mechanism of regulation of vitamin E status. *J. Biol. Chem.* 2002, *277*, 25290–25296.

[47] Sontag, T. J., Parker, R. S., Influence of major structural features of tocopherols and tocotrienols on their omega-oxidation by tocopherol-omega-hydroxylase. *J. Lipid Res.* 2007, *48*, 1090–1098.

[48] Mustacich, D. J., Leonard, S. W., Devereaux, M. W., Sokol, R. J., Traber, M. G., Alpha-tocopherol regulation of hepatic cytochrome P450s and ABC transporters in rats. *Free Radic. Biol. Med.* 2006, *41*, 1069–1078.

[49] Ohnmacht, S., Nava, P., West, R., Parker, R., Atkinson, J., Inhibition of oxidative metabolism of tocopherols with omega-N-heterocyclic derivatives of vitamin E. *Bioorg. Med. Chem.* 2008, *16*, 7631–7638.

[50] Hsu, M. H., Savas, U., Griffin, K. J., Johnson, E. F., Human cytochrome p450 family 4 enzymes: function, genetic variation and regulation. *Drug Metab. Rev.* 2007, *39*, 515–538.

[51] Hardwick, J. P., Cytochrome P450 omega hydroxylase (CYP4) function in fatty acid metabolism and metabolic diseases. *Biochem. Pharmacol.* 2008, *75*, 2263–2275.

[52] Kalsotra, A., Strobel, H. W., Cytochrome P450 4F subfamily: at the crossroads of eicosanoid and drug metabolism. *Pharmacol. Ther.* 2006, *112*, 589–611.

[53] Kikuta, Y., Kusunose, E., Kusunose, M., Characterization of human liver leukotriene B(4) omega-hydroxylase P450 (CYP4F2). *J. Biochem. (Tokyo)* 2000, *127*, 1047–1052.

[54] Liu, X., Zhao, Y., Wang, L., Yang, X. *et al.*, Overexpression of cytochrome P450 4F2 in mice increases 20-hydroxyeicosatetraenoic acid production and arterial blood pressure. *Kidney Int.* 2009, *75*, 1288–1296.

[55] Wu, J. H., Hodgson, J. M., Clarke, M. W., Indrawan, A. P. *et al.*, Inhibition of 20-Hydroxyeicosatetraenoic Acid Synthesis Using Specific Plant Lignans In Vitro and Human Studies. *Hypertension* 2009.

[56] Caldwell, M. D., Awad, T., Johnson, J. A., Gage, B. F. *et al.*, CYP4F2 genetic variant alters required warfarin dose. *Blood* 2008, *111*, 4106–4112.

[57] McDonald, M. G., Rieder, M. J., Nakano, M., Hsia, C. K., Rettie, A. E., CYP4F2 Is a vitamin K1 oxidase: An explanation for altered warfarin dose in carriers of the V433M variant. *Mol. Pharmacol.* 2009, *75*, 1337–1346.

[58] Jiang, Q., Freiser, H., Wood, K. V., Yin, X., Identification and quantitation of novel vitamin E metabolites, sulfated long-chain carboxychromanols, in human A549 cells and in rats. *J. Lipid Res.* 2007, *48*, 1221–1230.

[59] Freiser, H., Jiang, Q., Gamma-tocotrienol and gamma-tocopherol are primarily metabolized to conjugated 2-(beta-carboxyethyl)-6-hydroxy-2,7,8-trimethylchroman and sulfated long-chain carboxychromanols in rats. *J. Nutr.* 2009, *139*, 884–889.

[60] Freiser, H., Jiang, Q., Optimization of the enzymatic hydrolysis and analysis of plasma conjugated gamma-CEHC and sulfated long-chain carboxychromanols, metabolites of vitamin E. *Anal. Biochem.* 2009, *388*, 260–265.

[61] Christians, U., Transport proteins and intestinal metabolism: P-glycoprotein and cytochrome P4503A. *Ther. Drug Monit.* 2004, *26*, 104–106.

[62] Mustacich, D. J., Gohil, K., Bruno, R. S., Yan, M. *et al.*, Alpha-tocopherol modulates genes involved in hepatic xenobiotic pathways in mice. *J. Nutr. Biochem.* 2009, *20*, 469–476.

[63] Mustacich, D. J., Shields, J., Horton, R. A., Brown, M. K., Reed, D. J., Biliary secretion of alpha-tocopherol and the role of the mdr2 P-glycoprotein in rats and mice. *Arch. Biochem. Biophys.* 1998, *350*, 183–192.

[64] Kliewer, S. A., The nuclear pregnane x receptor regulates xenobiotic detoxification. *J. Nutr.* 2003, *133*, 2444S-2447S.

[65] Moore, J. T., Moore, L. B., Maglich, J. M., Kliewer, S. A., Functional and structural comparison of PXR and CAR. *Biochim. Biophys. Acta* 2003, *1619*, 235–238.

[66] Landes, N., Pfluger, P., Kluth, D., Birringer, M. *et al.*, Vitamin E activates gene expression via the pregnane X receptor. *Biochem. Pharmacol.* 2003, *65*, 269–273.

[67] Lehmann, J. M., McKee, D. D., Watson, M. A., Willson, T. M. *et al.*, The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J. Clin. Invest.* 1998, *102*, 1016–1023.

[68] Maglich, J. M., Stoltz, C. M., Goodwin, B., Hawkins-Brown, D. *et al.*, Nuclear pregnane x receptor and constitutive androstane receptor regulate overlapping but distinct sets of genes involved in xenobiotic detoxification. *Mol. Pharmacol.* 2002, *62*, 638–646.

[69] Rushmore, T. H., Kong, A. N., Pharmacogenomics, regulation and signaling pathways of phase I and II drug metabolizing enzymes. *Curr. Drug Metab.* 2002, *3*, 481–490.

[70] Dussault, I., Yoo, H. D., Lin, M., Wang, E. *et al.*, Identification of an endogenous ligand that activates pregnane X receptor-mediated sterol clearance. *Proc. Natl. Acad. Sci. USA* 2003, *100*, 833–838.

[71] Cho, J. Y., Kang, D. W., Ma, X., Ahn, S. H. *et al.*, Metabolomics reveals a novel vitamin E metabolite and attenuated vitamin E metabolism upon PXR activation. *J. Lipid Res.* 2009, *50*, 924–937.

[72] Brigelius-Flohe, R., Vitamin E and drug metabolism. *Biochem. Biophys. Res. Commun.* 2003, *305*, 737–740.

[73] Traber, M. G., Vitamin E, nuclear receptors and xenobiotic metabolism. *Arch. Biochem. Biophys.* 2004, *423*, 6–11.

[74] Leonard, S. W., Joss, J. D., Mustacich, D. J., Blatt, D. H. *et al.*, Effects of vitamin E on cholesterol levels of hypercholesterolemic patients receiving statins. *Am. J. Health Syst. Pharm.* 2007, *64*, 2257–2266.

[75] Werba, J. P., Cavalca, V., Veglia, F., Massironi, P. *et al.*, A new compound-specific pleiotropic effect of statins: Modification of plasma gamma-tocopherol levels. *Atherosclerosis* 2007, *193*, 229–233.

[76] Zhou, C., Tabb, M. M., Sadatrafiei, A., Grun, F., Blumberg, B., Tocotrienols activate the steroid and xenobiotic recep-

tor, SXR, and selectively regulate expression of its target genes. *Drug Metab. Dispos.* 2004, *32*, 1075–1082.

[77] Landes, N., Birringer, M., Brigelius-Flohé, R., Homologous metabolic and gene activating routes for vitamins E and K. *Mol. Aspects Med.* 2003, *24*, 337–344.

[78] Ichikawa, T., Horie-Inoue, K., Ikeda, K., Blumberg, B., Inoue, S., Steroid and xenobiotic receptor SXR mediates vitamin K2-activated transcription of extracellular matrix-related genes and collagen accumulation in osteoblastic cells. *J. Biol. Chem.* 2006, *281*, 16927–16934.

[79] Tabb, M. M., Sun, A., Zhou, C., Grun, F. *et al.*, Vitamin K2 regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. *J. Biol. Chem.* 2003, *278*, 43919–43927.

[80] Harrington, D. J., Soper, R., Edwards, C., Savidge, G. F. *et al.*, Determination of the urinary aglycone metabolites of vitamin K by HPLC with redox-mode electrochemical detection. *J. Lipid Res.* 2005, *46*, 1053–1060.

[81] Thijssen, H. H., Vervoort, L. M., Schurgers, L. J., Shearer, M. J., Menadione is a metabolite of oral vitamin K. *Br. J. Nutr.* 2006, *95*, 260–266.

[82] Tovar, A., Ameho, C. K., Blumberg, J. B., Peterson, J. W. *et al.*, Extrahepatic tissue concentrations of vitamin K are lower in rats fed a high vitamin E diet. *Nutr. Metab. (Lond)* 2006, *3*, 29.

[83] Booth, S. L., Golly, I., Sacheck, J. M., Roubenoff, R. *et al.*, Effect of vitamin E supplementation on vitamin K status in adults with normal coagulation status. *Am. J. Clin. Nutr.* 2004, *80*, 143–148.

[84] Traber, M. G., Siddens, L. K., Leonard, S. W., Schock, B. *et al.*, α-Tocopherol modulates Cyp3a expression, increases γ-CEHC production and limits tissue γ-tocopherol accumulation in mice fed high γ-tocopherol diets. *Free Radic. Biol. Med.* 2005, *38*, 773–785.

