Vitamin E revisited: do new data validate benefits for chronic disease prevention?

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Purpose of review

Vitamin E benefits in human health and chronic disease prevention are evaluated with respect to established α-tocopherol functions during vitamin E deficiency, adequacy, and excess.

Recent findings

Baseline vitamin E status of the 29 092 Finnish men participating in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention study showed that the men in the highest compared with the lowest quintile of serum α-tocopherol had significantly lower incidences of total and cause-specific mortality. New findings from the Women's Health Study support a role for vitamin E supplements in decreasing the risk for sudden death from cardiovascular disease and from thromboembolism. We speculate that a potential mechanism may involve vitamin E interference in vitamin K activation.

Summary

α-Tocopherol acts as a peroxyl and alkoxyl radical scavenger in lipid environments, and thus it prevents lipid peroxidation in lipoproteins and membranes, especially nervous tissues. Decreased chronic disease incidence is associated with lifelong generous dietary vitamin E intakes, but more than 90% of Americans do not consume the recommended dietary amounts (15 mg/day). Vitamin E supplements can have beneficial effects on health beyond those from dietary amounts, perhaps because pharmacologic levels also upregulate hepatic xenobiotic pathways.

Keywords

α-tocopherol, coronary heart disease, lipid peroxidation, neurological disease, xenobiotic metabolism

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Introduction

This review highlights α-tocopherol functions in humans with vitamin E deficiency, in those consuming 'optimal' dietary amounts, and in response to supplemental vitamin E. All of these responses are critical considerations in weighing the health benefits of α-tocopherol.

α-Tocopherol is a required nutrient

Vitamin E deficiency in humans occurs secondary to fat malabsorption, genetic defects in lipoprotein transport, or genetic defects in the hepatic α-tocopherol transfer protein (α-TTP) [1]. α-TTP transfers liver α-tocopherol into plasma lipoproteins for extrahepatic tissue delivery. Mechanisms by which α-TTP performs this action remain uncertain [2,3••].

Vitamin E deficiency symptoms have been observed in children during protein energy malnutrition [4,5]. Importantly from a clinical point of view, the symptoms could be reversed by vitamin E supplementation [5].

Human vitamin E deficiency symptoms

Symptoms of vitamin E deficiency are similar to those of Freidriech's ataxia. α-Tocopherol inadequacy causes a sensory neuropathy that is characterized by progressive dying back of large caliber axons in myelinated neurons [6]. Areflexia is observed first; after 3–5 years of vitamin E deficiency in children the entire constellation of neurologic abnormalities becomes apparent [7]. In adults it can take decades for deficiency symptoms to manifest [8]; frequently, no abnormalities during fat malabsorption syndromes can be directly related to low plasma α-tocopherol concentrations [9,10] because the initial presentation of 'loss of sensation' is mild and easily overlooked. Once progression of neurologic abnormalities is clinically significant, however, these abnormalities are often irreversible, although their further progression may be halted by vitamin E supplementation.

Anemia is a classic symptom of vitamin E deficiency in experimental animals, and erythrocyte fragility was used

as a vitamin E biomarker by the US Food and Nutrition Board [11]. Their recommendations are based on a study conducted in men consuming an experimental vitamin E-deficient diet in the 1950s, which used in-vitro susceptibility of erythrocytes to lysis after addition of hydrogen peroxide to assess the adequacy of vitamin E- repletion. Although chronic anemia during malnutrition may result from inadequate intakes of a variety of nutrients, anemia may also be caused by inadequate dietary vitamin E [12]. Increased vitamin E intake increases plasma α-tocopherol concentrations and decreases the incidence of anemia [13].

Increased incidences of chronic disease have not been reported in vitamin E deficiency. One explanation for this is that vitamin E deficiency often occurs in infants and in children rather than in adults, and thus chronic diseases would not be expected to occur. Vitamin E is an antioxidant, and so the concept that increased vitamin E intakes should be beneficial in ameliorating the oxidative stress observed in chronic disease continues to be a promising hypothesis, as discussed further below.

Optimal α-tocopherol intakes

The most recent recommended dietary allowance for vitamin E {15 mg [22 international units (IU) *RRR* or 33 IU *all rac*] α-tocopherol} was established in 2000 [11], but mean dietary intakes in the USA are only about 6 mg α-tocopherol [14•]. Importantly, 96% of American women and 93% of men do not meet current vitamin E recommendations [15]. The question is whether the recommendations are too high or whether dietary vitamin E consumption is too low.

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study determined whether supplementation for 5 years with vitamin E (50 IU-dl-α-tocopheryl acetate) and β-carotene (20 mg) would decrease cancer incidence [16]. The latest report from the ATBC study [17••] describes baseline vitamin E status in a cohort of 29 092 Finnish men who were followed for 19 years, during which time 13 380 deaths occurred. At baseline those in the highest compared with the lowest serum α-tocopherol quintile had significantly lower incidences of total and cause-specific mortality [relative risk (RR) 0.82, 95% confidence interval (CI) 0.78–0.86 for total mortality, and 0.79 (0.72–0.86), 0.81 (0.75–0.88) and 0.70 (0.63–0.79) for deaths due to cancer, cardiovascular disease, and other causes, respectively; P for trend for all < 0.0001]. The optimal reduction in mortality occurred at concentrations (30 μmol/l) associated with dietary intakes of about 13 mg α-tocopherol [17••]. Thus, a generous dietary vitamin E intake over a lifetime can decrease the incidence of chronic disease.

The Cache County study [18••] followed elderly participants for 7 years. In 1995, a total of 3831 Utah residents aged 65 years or older completed a baseline survey that included food frequency questionnaires and cognitive assessments. Participants in the highest quartiles of intake of dietary vitamins C and E scored higher on cognitive function tests and had a slower rate of cognitive decline over 3 years of follow up. Dietary vitamin E intake was about 16 IU/day, but supplements were also used by about 20% of participants. The benefit of increased vitamin E intakes for neurologic function is also supported by a 62% lower incidence of amyotrophic lateral sclerosis in regular users of vitamin E supplements, especially those taking supplements for longer than 10 years [19].

The Supplémentation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) study [20] used dietary levels of supplements in a randomized, double-blind, placebo-controlled primary prevention trial lasting about 7.5 years in France that involved 7876 women aged 35–60 years and 5141 men aged 45–60 years. The participants took a daily capsule containing either placebo or a combination of vitamin E (30 mg undefined source), ascorbic acid (120 mg), β-carotene (6 mg), selenium (100 μg), and zinc (20 mg). The supplementation lowered total cancer incidence and all-cause mortality in men (but not in women). The serum α-tocopherol concentration in the men was 35 μmol/l, which is consistent with observations in men who had beneficial effects of higher dietary vitamin E intakes at baseline in the ATBC trial. It is also interesting that more men ($n = 103$) than women ($n = 71$) died in the SU.VI.MAX study, suggesting that the men are at higher risk and therefore derived greater benefit from the antioxidant supplement.

Human circulating vitamin E concentrations in relationship to diet

Vitamin E is present at low concentrations in a variety of foods; therefore, estimates of dietary intakes are notoriously flawed. Vitamin E status assessed from circulating α-tocopherol concentrations apparently would be a better approach. α-Tocopherol is found in lipoproteins, however; therefore, as serum lipid levels increase, α-tocopherol and other vitamin E forms also increase [21]. Abnormalities in lipid metabolism that give rise to elevated circulating α-tocopherol may also interfere with delivery of α-tocopherol to tissues. This phenomenon was demonstrated in children with cholestatic liver disease who had elevated serum lipids and α-tocopherol, but had depleted tissue α-tocopherol and exhibited neurologic abnormalities [22]. Elevated serum α-tocopherol concentrations may also reflect high serum cholesterol, which is a predictor of increased risk for coronary heart disease [23]. Therefore, circulating α-tocopherol concentrations are

often reported per lipids, or lipid levels are taken into account in statistical models. Although circulating α-tocopherol concentrations may be associated with dietary vitamin E intakes [24], they also increase with age, along with lipids [25••]. Thus, elevations in circulating α-tocopherol concentrations are not necessarily an indicator of increased α-tocopherol intakes.

High-dose α-tocopherol supplements

'High-dose' vitamin E supplements may be defined as any amount of vitamin E that exceeds dietary levels [e.g. more than 15 mg (22 IU *RRR* or 33 IU *all rac*) α-tocopherol].

Randomized clinical trials to test vitamin E in at risk patients

The initial excitement about vitamin E supplements occurred about 15 years ago, when vitamin E supplements taken in amounts of 100 IU or more for 2 years or longer were found to be associated with decreased risk for coronary heart disease [26,27]. These observations were supported by a variety of mechanistic studies [28,29], and one of the first intervention studies with vitamin E demonstrated decreased risk for a second heart attack [30]. Subsequent trials with larger patient numbers did not demonstrate benefits of vitamin E in terms of decreasing heart disease risk [31,32]. Various meta-analyses have evaluated the outcomes of a number of vitamin E intervention trials in a variety of patient groups and have largely concluded that vitamin E has no benefit [33,34•,35••]; some even suggest that vitamin E supplements increase mortality [36]. Given these rather dire outcomes, why do we remain optimistic about vitamin E benefits in chronic disease risk prevention?

In general, in large randomized clinical trials of patients with various diseases, neither plasma α-tocopherol nor oxidative stress status was measured. These types of trials may not be the best for testing α-tocopherol efficacy because both experimental and 'placebo' groups consume dietary vitamin E. Patients who have adhered to appropriate dietary modifications to increase intakes of green leafy vegetables, 'healthy' fats, nuts and seeds, and consume whole grains, or those who are taking α-tocopherol in multivitamins are likely to have increased their dietary α-tocopherol intake to about 15 mg/day – an amount associated with decreased chronic disease mortality [17••].

To confound this issue further, α-tocopherol bioavailability is not necessarily optimal from vitamin E supplements and fortified foods. Using labeled vitamin E, Leonard *et al.* [37] showed plasma labeled α-tocopherol concentrations were higher following 30 IU consumed as a fortified cereal than following a 400 IU capsule taken without a fat-containing meal. Bruno *et al.* [38•] showed increased bioavailability with increasing amounts of fat consumed with the vitamin E. Unless circulating α-tocopherol is measured, it is difficult to determine whether there are differences in supplement and placebo groups, especially if the placebo group has plasma α-tocopherol concentrations above 30 μmol/l. In this case, it would be difficult to improve health further with vitamin E supplements. Thus, for a valid assessment of the reversal of symptoms by vitamin E supplements, it would be important to select individuals who have relatively low α-tocopherol and high oxidative stress status for inclusion in randomized clinical trials.

Vitamin E antioxidant effects

Recent studies of oxidative stress assessment have added to the confusion about vitamin E. When the relationship between vitamin E dose and suppression of lipid peroxidation in healthy humans was tested by providing vitamin E in daily amounts up to 2000 IU for 8 weeks, supplements had no effect on F_2-isoprostanes or 4-hydroxynonenal, which are lipid peroxidation biomarkers [39]. When vitamin E supplements (800 IU) were given to obese individuals for 3 months, no decrease in plasma F_2-isoprostanes was observed, but an increase in dose (1200 IU) for 3 months resulted in a decrease in plasma F_2-isoprostanes, without further elevation in α-tocopherol concentrations [40]. Roberts *et al.* [41•] tested vitamin E doses up to 3200 IU in hypercholesterolemic individuals given supplements for 16 weeks. They found a linear trend between percentage reduction in plasma F_2-isoprostanes and vitamin E doses, which reached significance at dosages of 1600 IU and 3200 IU. The largest dose, 3200 IU/day, caused a 49% suppression in plasma F_2-isoprostanes, 'which is not a profound reduction' and suggested that 'the antioxidant potency of vitamin E *in vivo* in humans is not great' [41•]. These decreases in plasma F_2-isoprostanes with vitamin E (1600 IU and more) may reflect increased F_2-isoprostane metabolism resulting from the pharmacologic amounts of α-tocopherol on hepatic xenobiotic metabolism (discussed below).

In contrast to the statements by Roberts *et al.* [41•], Traber and Atkinson [42•] speculated that α-tocopherol's antioxidant function is its only 'vitamin' function. α-Tocopherol does not prevent lipid peroxidation; it prevents the chain reaction that could occur when a peroxyl or alkoxyl radical is formed in a lipid milieu, such as a membrane, lipoprotein, or lipid droplet. When α-tocopherol intercepts a peroxyl radical, a lipid hydroperoxide is formed. F_2-isoprostanes are formed from the free radical-mediated oxidation of arachidonic acid; arachidonyl hydroperoxide is an intermediate in F_2-isoprostane formation [43,44]. Potentially, for every F_2-isoprostane formed, an α-tocopherol has intercepted a peroxyl radical. Thus, α-tocopherol antioxidant efficacy might

be estimated from lipid peroxidation biomarkers. The estimated urinary excretion of 2,3-dinor-5,6-dihydro-15-F(2t)-isoprostane (one of the F_2-isoprostane metabolites) is about 50 μg/day [45••], but this amount does not take into account other isoprostane metabolites or other fatty acids that might have been oxidized. Bayir *et al.* [46] estimated phospholipid oxidation in experimental brain injury and found that various phospholipids were differentially oxidized, with cardiolipin being especially sensitive (110 ± 20 pmol phospholipid hydroperoxide per nanomole phospholipid). None of these values is particularly informative with respect to vitamin E requirements, but they do emphasize that lipid peroxidation is not a rare event!

Evidence for in-vivo lipid peroxidation in healthy humans

To evaluate the role of vitamin E in ameliorating oxidative damage, Mastaloudis and coworkers [47,48] studied endurance exercisers who ran a 50 km ultramarathon race. The runners had increased plasma F_2-isoprostanes during the race and at its end; moreover, prior supplementation with antioxidants (400 IU vitamin E and 1000 mg vitamin C), as compared with placebo, prevented this increase. Vitamin E kinetics demonstrated increased rates of α-tocopherol disappearance during the race as compared with during a rest day [47]. Thus, the exercise depleted α-tocopherol and extra vitamin E prevented the increase in F_2-isoprostanes.

When α-tocopherol intercepts a peroxyl radical, an α-tocopheroxyl radical is formed whose most likely fate is to be reduced back to α-tocopherol by ascorbic acid [49]. When Bruno *et al.* [50] investigated α-tocopherol kinetics in cigarette smokers compared with nonsmokers, smokers had higher plasma α-tocopherol disappearance rates. Importantly, the rates were greater in smokers with low plasma ascorbic acid concentrations. Subsequently, Bruno *et al.* [51•] demonstrated that, when the individuals were taking placebo, α-tocopherol disappearance was faster in smokers than in nonsmokers, but with prior supplementation with vitamin C (500 mg twice daily) for 2 weeks the α-tocopherol disappearance rates in smokers decreased to rates observed in the nonsmokers (with or without vitamin C supplements). Importantly, there were no significant effects of vitamin C supplementation on plasma F_2-isoprostanes [51•,52].

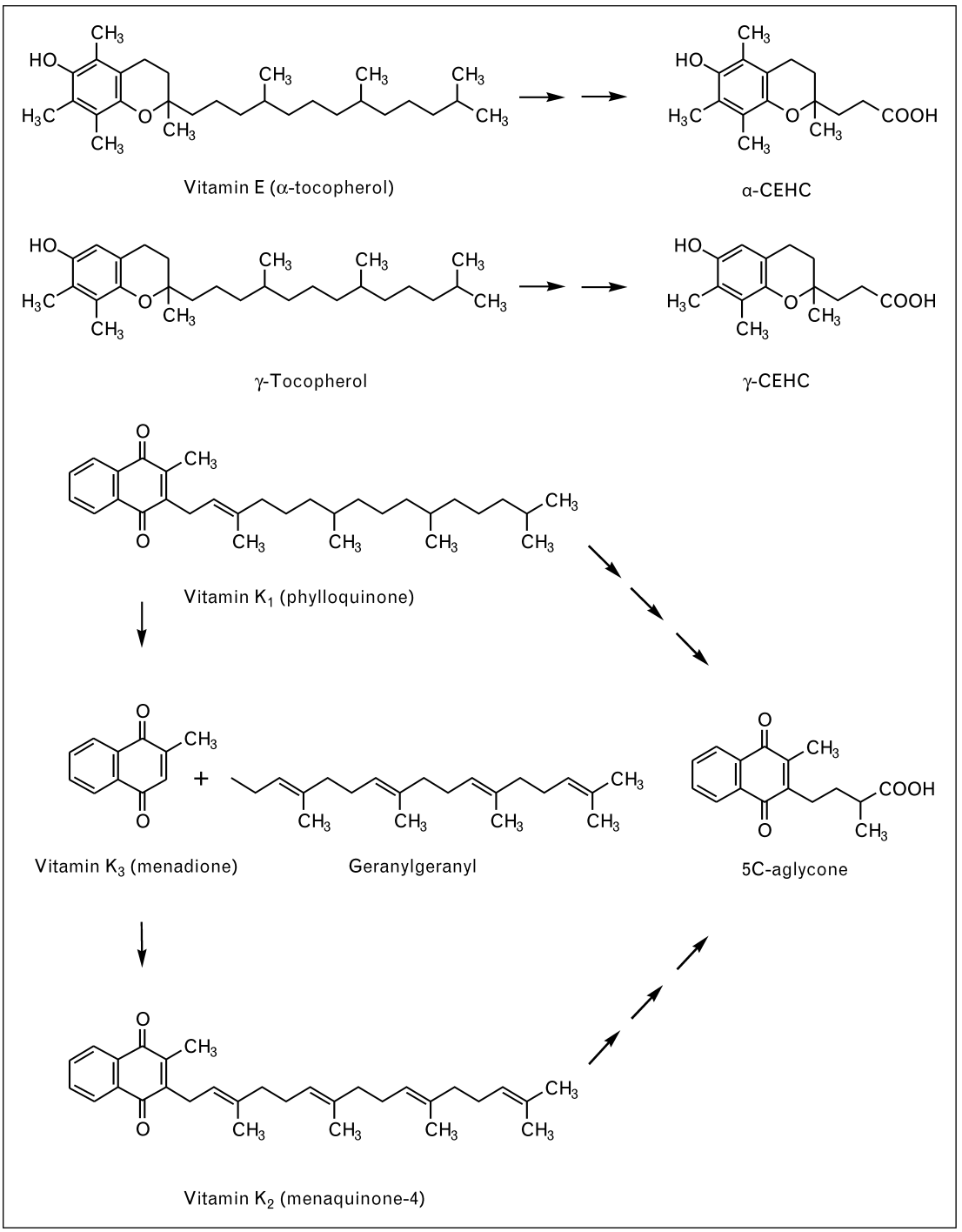
The oxidative stress imposed by cigarette smoking increased lipid peroxidation, but increasing ascorbic acid did not decrease plasma F_2-isoprostanes. These findings are consistent with the lack of change in plasma F_2-isoprostanes during vitamin C depletion and repletion studies in healthy women [53]. Thus, plasma F_2-isoprostanes reflect the inherent rate of radical production in lipid environments. α-Tocopherol does not halt the initial radical generation, but it limits the chain reaction of peroxyl radicals oxidizing neighboring polyunsaturated fatty acids. This hypothesis also provides an explanation for the simultaneous presence of α-tocopherol and oxidized lipids in human atherosclerotic lesions [54]. Polidori *et al.* [55], however, found that patients with intima media thickness greater than 0.5 mm have significantly higher levels of both F_2-isoprostanes and malondialdehdye concurrent with lower plasma α-tocopherol and carotenoids, despite the two groups having similar intakes of fruit and vegetable. This again suggests that atherosclerotic lesions are associated with increased oxidative stress. Thus, the prevention of oxidative stress and its sequelae is more likely to be successful than attempts to reverse existing damage.

The Women's Health Study

The Women's Health Study (WHS) [56] tested the efficacy of vitamin E supplements in preventing heart disease or cancer in healthy women. They evaluated 600 IU vitamin E or placebo taken every other day for 10 years in about 40 000 women aged 45 years and older. Overall, vitamin E supplements had no effect on incidence of cancer, cardiovascular events, or total mortality. The study also included secondary end-points, namely cardiovascular events, incidence of myocardial infarction, stroke, and cardiovascular death. Vitamin E supplements decreased deaths from cardiovascular disease by 24% (RR 0.76, 95% CI 0.59–0.98; $P = 0.03$). 'In subgroup analyses, women aged at least 65 years comprised 10% of study participants but contributed 31% of end points. A significant 26% reduction in major cardiovascular events was observed among women aged at least 65 years assigned to vitamin E (RR 0.74, 95% CI 0.59–0.93; $P = .009$) ... and 49% reduction in cardiovascular death (RR 0.51, 95% CI 0.33–0.77; $P < .001$) rates.' The decrease in cardiovascular death was attributed to a decrease in sudden death. Given that the incidence of cardiovascular disease is quite low in women until they are older than 65 years and that women lag behind men by 20 years with respect to sudden death (http://www.americanheart.org), the findings from the WHS suggest that vitamin E supplements are effective in decreasing death from cardiovascular disease.

The authors dismissed these secondary analyses as possible statistical artifacts, despite their assertion that corrections were made for post-hoc multiple comparisons [56]. In fact, they emphasized that 'It is possible that the observed reduction in cardiovascular deaths was due to chance, arising from multiple comparisons.' The WHS recently reported, however, that vitamin E supplementation decreased venous thromboembolism by 21% [57••]. Venous thromboembolism occurred in 482 women, 213 of whom were in the vitamin E group and 269 were in the placebo group (RR 0.79, 95% CI 0.66–0.94; $P = 0.010$).

Figure 1 Structures of vitamins E and K and their metabolites



Vitamin E metabolites [α–CEHC (2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman) and γ-CEHC (2,7,8-trimethyl-2-(2′carboxyethyl)-6-hydroxychroman)] are derived from α-tocopherol and α-tocotrienol and from γ-tocopherol and γ-tocotrienol, respectively. Vitamin E side chains first undergo ω-hydroxylation and then multiple rounds of β-oxidation. Vitamin K metabolites common to both phylloquinone and the menaquinone series are 2-methyl-3-(5′-carboxy-3′-methyl-2′-pentenyl)-1,4-naphthoquinone (7C-aglycone; not shown) and 2-methyl-3-(3′-3′-carboxymethylpropyl)-1,4-naphthoquinone (5C-aglycone). The vitamin K side chains first undergo ω-hydroxylation then multiple rounds of β-oxidation to yield the metabolites. Importantly, this hepatic metabolic process may produce menadione from phylloquinone. Menadione then can be converted to menaquinone-4 (vitamin K_2) by the addition of geranylgeranyl by the extrahepatic tissues.

Although atherosclerosis is not associated with increased incidence of thromboembolism [58•], the latest findings from the WHS do provide a possible mechanistic basis for the decreased cardiovascular mortality observed with vitamin E supplements. Both decreased sudden death and decreased thromboembolism may arise from α-tocopherol's pharmacologic effects, which result in decreased clot formation.

Xenobiotic metabolism and vitamin E supplements

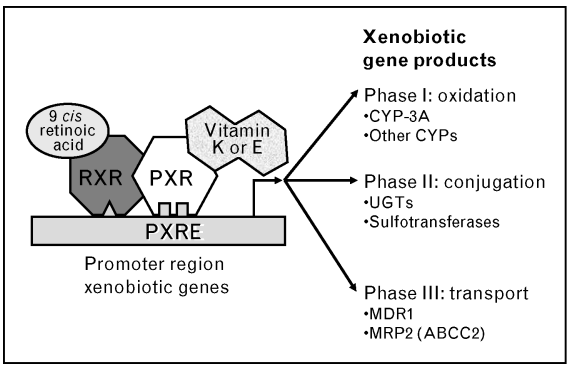
For over 30 years, α-tocopherol has been recognized to decrease platelet aggregation [59], a process that was subsequently shown to occur through a protein kinase C-dependent mechanism [60–62]. High α-tocopherol concentrations were required for this in-vitro effect, however. Based on new studies of vitamins E and K metabolism, we propose that the beneficial effect of vitamin E on sudden death and decreased thromboembolism observed in the WHS could result from an interaction between vitamins E and K metabolism (Fig. 1). These two fat-soluble vitamins appear to have overlapping mechanisms that both regulate vitamin E concentrations and activate vitamin K – a nutrient that is involved in blood clotting and formation of γ-carboxyglutamic acid in various proteins. Not only do their metabolic pathways overlap, but the two vitamins also both bind to nuclear receptors that regulate the same xenobiotic pathways (Fig. 2).

Hepatic vitamin E concentrations are regulated by metabolism [63•,64]; the side chain is ω-hydroxylated and then β-oxidized, forming metabolites (hydroxychromans; Fig. 1) [65,66]. The initial ω-hydroxylation is mediated by cytochrome P450 (CYP)-4F2 (or tocopherol hydroxylase) [66,67••]. Tocopherol hydroxylase has a preference for vitamin E forms without a 5-methyl group (e.g. γ-tocopherol) and/or an unsaturated side chain [67••]. Labeled γ-tocopherol administration to humans revealed rapid metabolism, whereas α-tocopherol was metabolized only to a limited extent [68]. α-Tocopherol metabolism increased with increasing vitamin E dose, however [69]. Noteably, CYP-4F forms can metabolize arachidonic acid, prostaglandins, and leukotrienes [70•].

Although the initial ω-hydroxylation of the vitamin E side chain is mediated by CYP-4F2 [66,67••], elevated hepatic α-tocopherol upregulates CYP-3A and multidrug resistance protein (Fig. 2) but not CYP-4F2, as shown in rodent studies [63•,64,71•]. The orphan nuclear receptor pregnane xenobiotic receptor (PXR; also known as SXR), along with the constitutive androstane receptor, have overlapping abilities to regulate xenobiotic detoxifying enzymes and transporters such as CYP-3A4 and multidrug resistance protein (Fig. 2) [72]. PXR and constitutive androstane receptor are activated by ligand binding; various vitamin E forms are ligands for PXR [73,74]. Additionally, vitamin K is a PXR ligand [75]. PXR regulates hepatic xenobiotic pathways, but in osteoblasts it regulates extracellular matrix and collagen formation [76•,77]. Moreover, vitamins E and K are both ω-hydroxylated and then undergo β-oxidation [78,79••].

More than 90% of dietary, plant-derived vitamin K is phylloquinone (vitamin K1; Fig. 1) [78]. Other vitamin Ks are menaquinones, which can have multiple prenyl side chains, as indicated by their suffix number (i.e. menaquinone-n). The liver converts phylloquinone to menadione, which is then used by extrahepatic tissues for menaquinone-4 synthesis [80•]. Hypothetically, vitamin E interferes with the conversion to menadione because extrahepatic tissue menaquinone-4 concentrations are lower in rats fed a diet high in vitamin E [81]. Additionally, high-dose vitamin E supplementation in humans (1000 IU) increased a biomarker of inadequate vitamin K status [82]. Also, vitamins E and K appear to share a pathway for urinary metabolite excretion. Vitamin K metabolite (5C-aglycone; Fig. 1) is common to both phylloquinone and menaquinone, and Harrington *et al.* [79••] showed, during a controlled metabolic study, that 5C-aglycone represents 75% of phylloquinone excretion.

Figure 2 Scheme of the effects of ligand binding to pregnane xenobiotic receptor



Hypothetically, vitamins E and K could bind to the same ligand-binding site of pregnane xenobiotic receptor (PXR). When PXR dimerizes with retinoid X receptor (RXR) with bound retinoic acid, the dimer can then bind to the PXR response element (PXRE) in the promoter region of various xenobiotic metabolism-related proteins (phase I, II, or III). In this manner, excess vitamins E and K can regulate their own metabolism. We postulate that vitamin E supplements may increase hepatic α-tocopherol to sufficiently high levels as to interfere not only with phylloquinone binding to PXR but also with the conversion of phylloquinone to menaquinone-4 by preventing the phylloquinone tail shortening (see Fig. 1 for structures). Presumably, α-tocopherol would competitively prevent the ω-hydroxylation of the tail and its subsequent β-oxidation. The net result would be a decrease in the active vitamin K_2 form (menaquinone-4). ABCC2, ATP-binding cassette, subfamily C, member 2; MDR1, multidrug resistance; MRP2, multidrug resistance protein 2; UGT, UDP-glucuronosyltransferase.

We speculate that the benefits of vitamin E supplementation observed in the WHS were in part due to antioxidant effects of α-tocopherol. In addition, vitamin E acts as an antithrombotic agent, decreasing the likelihood of clot formation. Vitamin E excess causes an increased tendency to bleed [11], and the observed adverse effect in the WHS [56,57••] was an increase in nose bleeds (epistaxis RR 1.06, 95% CI 1.01–1.11; $P = 0.02$).

Conclusion

α-Tocopherol is necessary to prevent propagation of lipid peroxidation [42•], but more than 90% of the US population does not consume an amount (15 mg or 22 IU) [15] that decreases chronic disease risk [17••]. Outcomes from the WHS suggest that vitamin E supplements are beneficial when they are taken before disease onset, but vitamin E and K interactions may be significant in these health outcomes. The negative evidence regarding vitamin E supplements from randomized clinical trials is more a reflection of inadequate study design and methods of analysis than proof of failure of vitamin E in primary prevention.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 75–76).

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