Vitamin E and K interactions – a 50-year-old problem

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The mechanisms by which vitamin E interferes with vitamin K activity, especially blood clotting, are not known, but hypothetically this interference may involve metabolic pathways. Phylloquinone (K\textsubscript{1}) must be converted to menaquinone (MK-4, the most potent extrahepatic tissue vitamin K) by truncation of the K\textsubscript{1} side chain and replacement with geranylgeranyl. Possible mechanisms for the vitamin E and K interaction include: 1) vitamin E competes for the yet undiscovered enzyme that truncates the K\textsubscript{1} side chain; 2) vitamin E competes with K\textsubscript{1} for the hypothetical cytochrome P450 enzyme that hydroxylates the K\textsubscript{1} side chain, thereby preventing its \( \beta \)-oxidation and its removal for MK-4 formation; or 3) vitamin E increases xenobiotic pathways that increase hepatic metabolism and excretion of all vitamin K forms. Currently, the pathway for K\textsubscript{1} conversion to MK-4 is unknown, the process for regulating vitamin K metabolism to urinary excretion products is unknown, and why vitamin E supplements have such a dramatic effect, causing bleeding in some individuals and not in others, remains a mystery.

INTRODUCTION

Vitamin E and K interactions have been recognized for over 50 years, yet the mechanisms for this interaction are unknown. In 2000, the US-based Food and Nutrition Board set the vitamin E upper tolerable limit (UL) for humans based on studies in rats.\textsuperscript{1} Specifically, high vitamin E intakes in rats caused increased bleeding, a phenomenon that could be reversed by increased vitamin K administration.\textsuperscript{2} Recent reports from the Women’s Health Study demonstrate that vitamin E supplements decrease the risk of mortality from thromboembolism;\textsuperscript{3} thus, \( \alpha \)-tocopherol decreases the tendency for blood to clot in normal healthy women. In addition, vitamin E supplements in humans increase under-carboxylation of prothrombin,\textsuperscript{4} again suggesting vitamin E decreases vitamin K status. Clearly, vitamin E supplements have marked effects on human vitamin K status that are not well understood.

Currently, the biochemical mechanisms for conversion from phylloquinone (K\textsubscript{1}) to menaquinone-4 (MK-4) and for regulating metabolism of vitamin K forms to urinary excretion products are unknown. Moreover, how vitamin E supplements decrease thrombosis is unknown. The purpose of this review is to present some possible explanations for how vitamin E interferes with vitamin K status.

CLINICAL STUDIES SHOWING VITAMIN E AND K INTERACTIONS

The Women’s Health Study tested the efficacy of vitamin E supplements to prevent heart disease or cancer in normal healthy women.\textsuperscript{5} Either vitamin E (600 IU, RRR-\( \alpha \)-tocopheryl acetate) or placebo was taken every other day for 10 years by about 40,000 normal, healthy women, aged 45 years and older. Overall, the authors concluded that vitamin E supplements had no effect on cancer incidence, cardiovascular disease, or total mortality.\textsuperscript{3} The study also included secondary endpoints: 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 endpoints. Among women aged at least 65 years assigned to vitamin E, a significant 26% reduction in major cardiovascular events was observed (RR, 0.74; 95% CI, 0.59–0.93; \( P = 0.009 \)), and cardiovascular death rates were reduced by 49% (RR, 0.51; 95% CI, 0.33–0.77; \( P < 0.001 \)). The decreased incidence of cardiovascular death in the vitamin E group was attributed to a decrease in sudden death, but no mechanism was suggested.5

Given that the incidence of cardiovascular disease is quite low in women until they are over the age of 65 years and that women lag behind men by 20 years with respect to sudden death (http://www.americanheart.org), the findings from the Women’s Health Study suggest that vitamin E supplements are effective in decreasing the incidence of sudden death, potentially because they decrease blood clot formation.6 Importantly, a follow-up publication from the Women’s Health Study showed that vitamin E supplementation decreased venous thromboembolism by 21%.3 Venous thromboembolism occurred in 482 women: 213 in the vitamin E group and 269 in the placebo group (RR, 0.79; 95% CI, 0.66–0.94; \( P = 0.010 \)). As emphasized by the Women’s Health Study authors,3 atherosclerosis is not associated with increased incidence of thromboembolism.7

Importantly, the adverse effect caused by excess vitamin E was described by the Food and Nutrition Board, based on studies in rats, as an increased tendency to bleed.1 Large \( \alpha \)-tocopherol supplements in the Women’s Health Study acted as a mild anti-thrombotic agent, thereby decreasing the likelihood of clot formation.3 The adverse effect of vitamin E observed in the Women’s Health Study was an increase in nose bleeds (epistaxis RR, 1.06; 95% CI, 1.01–1.11; \( P = 0.02 \)).3,5 Based on new studies of vitamin K metabolism,8,9 we propose that both the beneficial and the adverse effects of large \( \alpha \)-tocopherol intakes could be due to an interaction between vitamin E upregulating xenobiotic metabolism and/or vitamin K activation or metabolism.

**VITAMIN K ACTIVATION AND METABOLISM**

With its 20 carbon, phytol side chain, vitamin K\(_1\) constitutes more than 90% of dietary, plant-derived vitamin K (Figure 1).8 Other vitamin K forms are the menaquinones (MK), which have multiple prenyl side chains, as indicated by their suffix number (i.e., MK-n). Importantly, \( K_1 \) must be converted to MK-4 by (partial?) truncation of its phytol tail, and tail replacement with geranylgeranyl (Figure 1).10

\( K_1 \) is converted to menadione, which is then used by extra-hepatic tissues for MK-4 synthesis.11 Okano et al.10 recently demonstrated this pathway in mice using deuterium-labeled vitamin K forms. However, they emphasized that the site and the mechanism for the conversion remain unknown. They suggested the intestine was a likely site of \( K_1 \) conversion to menadione because oral (but not injected) \( K_1 \) was converted to MK-4. However, \( K_1 \) is fat soluble and is transported in chylomicrons to the liver, as reviewed by Kohlmeier et al.12 Potentially, chylomicron remnants deliver \( K_1 \) to the liver where side chain removal could also occur. Thus, it seems plausible that the injected vitamin K in the Okano et al.10 study was not in a readily bioavailable form. Currently, the site of \( K_1 \) conversion to MK-4 remains controversial.

![Figure 1 Structures of vitamins E and K and their metabolites.](image)

Phylloquinone (\( K_1 \)), menaquinone (MK-4), and \( \alpha \)-tocopherol are shown. The vitamin E metabolite is \( \alpha \)-CEHC [2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxycroman]. Vitamin E and K side chains, hypothetically, first undergo \( \omega \)-hydroxylation, then multiple rounds of \( \beta \)-oxidation to form these metabolites. Vitamin K metabolites common to both \( K_1 \) 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 K vitamins are metabolized to the 5C-aglycone (Figure 1). This vitamin K metabolite is common to both K1 and MK-4. Both are thought to undergo a process of side chain \( \omega \)-hydroxylation and \( \beta \)-oxidation during their metabolism.\(^9\) Harrington et al.\(^9\) showed that the 5C-aglycone represents 75% of K1 excreted during a controlled metabolic study.

**VITAMIN E EXCESS AND XENOBIOTIC METABOLISM**

Dietary components with vitamin E antioxidant activity include \( \alpha \)-, \( \beta \)-, \( \gamma \)-, and \( \delta \)-tocopherols and \( \alpha \)-, \( \beta \)-, \( \gamma \)-, and \( \delta \)-tocotrienols. These molecules all have a chromanol ring and vary in the number of methyl groups on the chromanol ring. Tocopherols have a phytol side chain and tocotrienols have unsaturated side chains. All the various vitamin E forms are absorbed, transported in chylomicrons, and following a single pass in the circulation, chylomicron remnants deliver these antioxidants to the liver, as reviewed previously.\(^14\) The liver expresses the \( \alpha \)-tocopherol transfer protein, which facilitates the preferential secretion of \( \alpha \)-tocopherol into plasma.\(^14\)

Hepatic vitamin E forms and concentrations are regulated in large part by metabolism.\(^15,16\) Initially, the vitamin E side chain is \( \omega \)-hydroxylated, then \( \beta \)-oxidized to form carboxy ethyl hydroxy chromanols, CEHCs (Figure 1).\(^17,18\)

Vitamins E and K appear to share the same metabolic pathways because both undergo side chain \( \omega \)-hydroxylation and \( \beta \)-oxidation to produce their respective metabolites. The cytochrome P450s (CYPs) involved in vitamin E metabolism have been investigated by Sontag and Parker.\(^18\) They identified that only CYP4F2 was involved in the \( \omega \)-oxidation of \( \gamma \)-tocopherol and called this enzyme, tocopherol hydroxylase.\(^18\) To date, no similar studies to define the CYP enzymes involved in the \( \omega \)-oxidation of the K1 and MK-4 side chains, respectively, have been reported.

Although tocopherol hydroxylase (CYP4F2) has a preference for the vitamin E forms without a 5-methyl group (e.g., \( \gamma \)-tocopherol) and/or with an unsaturated side chain (e.g., tocotrienols),\(^19\) it is not clear that CYP4F2 is the only mechanism for \( \omega \)-hydroxylation of the phytol side chain of the various vitamin E forms. We have shown that CYP4F2 protein concentrations are not responsive to hepatic \( \alpha \)-tocopherol concentrations.\(^15,16,20\)

Moreover, there is likely overlap in ligand specificity between CYPs because CYP3A family members also have been suggested to be involved in vitamin E metabolism.\(^21–24\)

**POSSIBLE MECHANISMS FOR THE VITAMIN E AND K INTERACTION**

As illustrated in Figure 2, potential mechanisms for the vitamin E and K interactions include: 1) vitamin E competes for the enzyme (yet undiscovered) that truncates the K1 side chain to form menadione; or 2) vitamin E increases xenobiotic pathways\(^15,20\) that increase the metabolism and excretion of all vitamin K forms from the liver; or 3) vitamin E competes with K1 for the cytochrome P450 (CYP) that \( \omega \)-hydroxylates the tail, thereby preventing the \( \beta \)-oxidation of the tail to form menadione. The latter mechanism is hypothetically less likely because,
as discussed by Thijssen et al., there is likely to be steric hindrance preventing the complete removal of the K side chain via β-oxidation for menadione formation. All of these mechanisms would decrease the production and/or availability of MK-4.

The hypothesis that vitamin E interferes with the conversion of K to MK-4 is supported by the observations that MK-4 concentrations in extrahepatic tissue are lower in rats fed a high vitamin E diet. In humans, high-dose vitamin E supplements (1000 IU) increased a biomarker of inadequate vitamin K status, i.e., the under-gamma-carboxylated prothrombin (proteins induced by vitamin K absence-factor II, PIVKA-II). Additionally, coagulation factor IX (FIX) is altered by vitamin E status. FIX is synthesized in the liver where vitamin K-dependent carboxylation of glutamate (Gla) residues of FIX and other specific proteins takes place. Possibly, vitamin E directly antagonizes vitamin K by preventing formation of Gla-proteins. Indeed, high vitamin E intakes in rats decreased gamma-carboxylation of prothrombin, suggesting lower vitamin K activity, but plasma K concentrations were unchanged.

Other mechanisms for the interaction of these two vitamins have been proposed. Dowd and Zheng demonstrated that α-tocopheryl quinone, but not α-tocopherol, is a potent inhibitor of vitamin K-dependent carboxylase. However, since α-tocopheryl quinone concentrations 1) are extremely low in vivo, 2) increase with oxidative stress, and 3) are likely to decrease with vitamin E supplementation, the interaction of α-tocopheryl quinone with the vitamin K-dependent carboxylase is unlikely to be the explanation for increased bleeding during vitamin E supplementation.

An alternative explanation for the decreased blood clotting could be α-tocopherol’s effect on platelets. For over 30 years, vitamin E effects on platelet function have been studied. Specifically, α-tocopherol has been recognized to decrease platelet aggregation, a process that was subsequently shown to occur through a protein kinase C-dependent mechanism. However, these findings were largely based on in vitro incubations, and high α-tocopherol concentrations in the incubation medium were required for the observed effects. Thus, it seems less likely that platelet effects adequately encompass the α-tocopherol antithrombotic effects.

**ARE XENOBIOTIC PATHWAYS THE SITE OF VITAMIN E AND K INTERACTIONS?**

Possible mechanisms by which vitamin E and K could interact include various xenobiotic pathways. Our studies examining the mechanisms by which the liver prevents vitamin E over-accumulation have suggested that α-tocopherol induces a coordinated increase in various detoxification systems. Specifically, increased liver α-tocopherol concentrations in rats that had been injected subcutaneously with vitamin E caused an upregulation in hepatic concentrations of CYP3A, CYP2B, and CYP2C. Elevated hepatic α-tocopherol also upregulated multi-drug resistance (MDR1) protein. Dietary as well as injected vitamin E caused both upregulation of CYP3A and a coordinated regulation of xenobiotic genes (unpublished data, Mustacich D, et al.). Specifically, gene expression of the phase I enzymes P450 oxidoreductase and Cyp3a11 increased 1.6- and 4.0-fold; phase II genes, sulfotransferase 2a and glutathione S-transferase μ3, increased 10.8- and 1.9-fold; and a phase III biliary transporter, Mdr1a, doubled (unpublished data, Mustacich D, et al.). Thus, consumption of excess dietary α-tocopherol by mice coordinately upregulated genes involved in xenobiotic metabolism.

Hypothetically, high α-tocopherol intakes could interfere with vitamin K status because vitamin E supplementation increases CYP3A, which is involved in metabolism of 50% of prescription drugs (as well as other dietary components), and upregulated MDR-1, a transporter that enhances hepatic excretion of lipophilic compounds into bile. These upregulated xenobiotic pathways could degrade vitamin K status because they would further increase the usual rapid hepatic vitamin K turnover, a phenomenon that was demonstrated in the 1980s by Kindberg and Suttie.

Xenobiotic metabolism is under the control of nuclear receptors. The nuclear receptors, pregnane xenobiotic receptor (PXR, also known in humans as SXR) and constitutive androstane receptor (CAR), have overlapping abilities to regulate various xenobiotic detoxifying enzymes and transporters, including CYP3A4 and MDR1. Both PXR and CAR are activated by ligand binding; various vitamin E forms, especially tocotrienols with their unsaturated tails are ligands for PXR. Importantly, MK-4 with its unsaturated tail is a PXR ligand.

PXR regulates xenobiotic pathways in the liver; however, with respect to bone formation, PXR with bound MK-4 in osteoblasts regulates extracellular matrix and collagen formation. Given that the regulatory function of MK-4 in bone is mediated by PXR, it is possible that in the liver, MK-4 metabolism is also modulated by PXR. Hypothetically, both vitamins E and K could bind to PXR. This suggestion opens the possibility that binding of vitamin E forms by PXR competes with MK-4 binding and interferes with hepatic regulation of vitamin K metabolism by PXR. Hypothetically, vitamin E supplements may increase hepatic α-tocopherol to sufficiently high levels so as to stimulate PXR and thereby promote the metabolism and excretion of K1 and MK-4.
CONCLUSION

In summary, vitamins E and K appear to share the same metabolic pathways; the side chain of both are ω-hydroxylated, then undergo β-oxidation. Hypothetically, MK-4 is decreased in response to high hepatic α-tocopherol and, therefore, the most active form of vitamin K, MK-4, is depleted.

There are various possible mechanisms that could account for the decreased MK-4 concentrations. Vitamin E may interfere with formation of MK-4 from K1. K1 must account for the decreased MK-4 concentrations. Vitamin vitamin K, MK-4, is depleted.

Vitamin E may increase metabolic pathways and deplete all vitamin K forms. Clearly, more studies are needed.

REFERENCES

28. Gregor W, Staniek K, Nohl H, Gille L. Distribution of tocopherol quinone in mitochondrial membranes and interfer-