Vitamin E Revisited
Maret Traber, PhD

Mechanism of action
The controversy: vitamin E's benefits in human health and chronic disease prevention
Potential mechanisms for vitamin E's benefits

Vitamin E is a Required Nutrient
- Vitamin E is present in foods
- Vitamin E is detectable in all human tissues
- Vitamin E deficiency symptoms
  - occur as a result of inadequate tissue concentrations
  - can be reversed by vitamin E

What are Human Vitamin E Deficiency Symptoms?
- Peripheral neuropathy
- Loss of sensation in hands & feet
- Inability to walk--ataxia

What causes vitamin E deficiency in humans?
- Almost never as a result of limited dietary vitamin E
- Fat malabsorption
  - Cholestatic liver disease
  - Cystic fibrosis
  - Abnormalities in lipoprotein synthesis
- Genetic abnormalities in α-tocopherol transfer protein (α-TTP)

Clinical Features and Associated Neuromuscular Lesions in Humans with Vitamin E Deficiency
- Loss of Position and Vibratory Sensation
  - Peripheral Nerve
  - Posterior Columns
- Ataxia
  - Cerebellum
  - Spinocerebellar tracts
- Weakness
  - Skeletal Muscle
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Food Sources of Vitamin E

- Vitamin E status in the cohort of 29,092 Finnish men was followed for 19 years; 13,380 deaths ensued.
- At baseline those in the highest compared with the lowest serum α-tocopherol quintile had significantly lower incidences of:
  - total mortality; relative risk (RR) = 0.82 (95% CI: 0.78, 0.86)
  - cause-specific mortality: cancer: 0.79 (0.72, 0.86), cardiovascular disease 0.81 (0.75, 0.88), other 0.70 (0.63, 0.79)
  - P for trend for all < 0.0001
- Optimum reduction in mortality occurred at serum concentrations (30 μmol/L) associated with dietary intakes of ~13 mg α-tocopherol

Most people do not consume the average required amount of vitamin E (EAR = 12 mg α-tocopherol/day)

Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study

- Women who took vitamin E supplements for more than two years had a relative risk of major coronary disease of 0.59 (95 percent confidence interval, 0.38 to 0.91)
  - Stampfer, MJ NEJM 328:1444, 1993

Vitamin E Functions in Heart Health

- Protects LDL from oxidation
- Decreases artery wall inflammation
- Reduces platelet aggregation or clotting
- Decreases adhesion of platelets and white blood cell to artery walls
- Promotes artery wall flexibility

Vitamin E Consumption and Risk of Coronary Disease in Women

- 87,245 female nurses 34 to 59 years old
  - Follow-up of up to eight years (679,485 person-years)
    - 552 cases of major coronary disease
    - 437 nonfatal myocardial infarctions
    - 116 deaths due to coronary disease
- Women who took vitamin E supplements for more than two years had a relative risk of major coronary disease of 0.59
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JAMA Feb 28, 2007

Conclusions: Treatment with beta-carotene, vitamin A and vitamin E may increase mortality. The potential roles of vitamin C and selenium on mortality need further study.

JAMA. 2008;300(18):2123-2133

Conclusions In this large, long-term trial of male physicians, neither vitamin E nor vitamin C supplementation reduced the risk of major cardiovascular events. These data provide no support for the use of these supplements for the prevention of cardiovascular disease in middle-aged and older men.

JAMA. 2009;301(1):52-62

Conclusions In this large, long-term trial of male physicians, neither vitamin E nor C supplementation reduced the risk of prostate or total cancer. These data provide no support for the use of these supplements for the prevention of cancer in middle-aged and older men.

JAMA. 2009;301(1):39-51

Conclusion Selenium or vitamin E, alone or in combination at the doses and formulations used, did not prevent prostate cancer in this population of relatively healthy men.

Vitamin E in Heart Disease Prevention —Women’s Health Study

- 40,000 women aged 45 y and older randomly assigned:
  - vitamin E (600 IU every other day) or placebo
  - aspirin or placebo
  - study lasted 10 y
- 24% reduction in cardiovascular death
  - largely attributable to fewer sudden deaths in the vitamin E group (38 vs. 51 in the placebo group)
- No reduction in stroke rate was observed
- No effect of vitamin E on total mortality
Lee et al., JAMA 294, 56-65 (2005).

Vitamin E in Heart Disease Prevention—Women’s Health Study Subgroup Analysis

- In women aged at least 65 y (10% of study participants) assigned to vitamin E:
  - 26% reduction in major cardiovascular events
  - 34% reduction in myocardial infarction
  - 49% reduction in cardiovascular deaths
- Vitamin E efficacy was not evaluated with biomarkers, but with mortality or heart attacks, etc.
- Study authors concluded that vitamin E provided no overall benefit and do not support recommending vitamin E supplementation for cardiovascular disease prevention among healthy women.
Lee et al., JAMA 294, 56-65 (2005).
WHS reported that vitamin E supplementation decreased venous thromboembolism by 21%.
Venous thromboembolism occurred in 482 women: 213 in the vitamin E group and 269 in the placebo group. \[RR, 0.79; 95\% CI, 0.66 to 0.94; P=0.010\].
Both decreased sudden death and decreased thromboembolism may arise from \(\alpha\)-tocopherol's pharmacologic effects that result in decreased clot formation.
Glynn et al., Circulation 116, 1497-503, 2007

Vitamin E is an antithrombotic agent, decreasing platelet aggregation.
Vitamin K is involved in the blood clotting cascade.
Vitamin E and K interactions have been recognized for >50 years, but the mechanisms for the interaction are still unknown.

Hypothesis: beneficial vitamin E effect in thrombosis could result from an interaction with vitamin K metabolism, decreasing the activation of vitamin K.

Vitamin K and E structures

Vitamin K activation
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Both decreased sudden death and decreased thromboembolism may arise from α-tocopherol’s pharmacologic effects that result in decreased clot formation.

Vitamin E & K interactions
- Vitamin E is an antithrombotic agent, decreasing platelet aggregation
- Vitamin K is involved in the blood clotting cascade
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Vitamin K and E structures

Vitamin K activation
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Hypothesis

- Vitamin E (α-tocopherol) interferes with formation of the most potent extrahepatic tissue vitamin K form, menaquinone-4 (MK-4)

What factors explain vitamin E’s lack of adverse effects?

- Vitamin E Metabolism
- Interactions with xenobiotic metabolism

What is a Xenobiotic?

- Xenobiotic: chemical compound that is foreign to the body of a living organism.
  - Drugs, pesticides, vitamins, herbs
  - Any compound not synthesized by the body itself

Vitamin E and Nuclear Receptors

- Vitamin E is a ligand for the pregnane X receptor (PXR) (Landes et al. ’03)
- PXR:
  - an orphan nuclear receptor
  - regulates a variety of xenobiotic metabolizing enzymes
  - binds to the response element in the human CYP3A4 promoter
  - is activated by a range of drugs known to induce CYP3A4 expression (LeCluyse, ’01, Lehmann et al. ’98)
- CYP3A4 metabolizes more than 50% of drugs (Kliewer et al ’02)

Postulated Gene Regulation by Vitamin E binding to PXR

Vitamin E

- α-tocopherol has the highest biologic activity
  - preferentially retained in plasma and tissues
- γ-tocopherol
  - predominant dietary form
  - actively metabolized
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Deuterated α- and γ-Tocopherols

Deuterated Tocopherol Concentrations in a Representative Subject: Actual Data Compared with Fitted Line

α-Tocopherol is more effectively retained in the plasma

Fractional Disappearance Rate (pools per day) and Half-Life (hours)

Fractional Disappearance Rate

γ-Tocopherol and its metabolite disappear rapidly from plasma

Regulation of Liver Vitamin E

Preferential Secretion of α-Tocopherol Mediated by Hepatic α-Tocopherol Tocopherol Transfer Protein


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Altered gene expression in mice consuming 1000 IU vs 30 IU dl-α-tocopherol

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Mouse Real-time PCR Protocol

- Real-time PCR
  - RT2 Primer sets were purchased from Superarray
  - Efficiency of each primer set was determined using serial dilutions of reference RNA and RNA from control and supplemented mice
  - Expression of the gene of interest was normalized to expression of a house-keeping gene
    - Hypoxanthine guanine phosphoribosyl transferase 1 (HPRT)
  - Fold-change determined by calculating $2^{-\Delta\Delta Ct}$
  - Genes changed ≥ 1.5-fold with $P < 0.05$ were considered significant

Xenobiotic Metabolism in Rats Modulated by Vitamin E Given as Subcutaneous Injections

Protocol

- Rats injected α-T daily s.c.
  - 10 mg/100g body wt., Vital-E 330, Schering-Plough
- Livers were collected on every third day, measurements included:
  - Both α-T and α-CEHC
  - CYP proteins in microsomal fractions
  - MDR proteins in total lysates

Hepatic α-T and α-CEHC Decrease Despite Daily α-T Injections

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Hepatic CYP3A increases, while CYP4F protein levels remain unchanged

Samples expressed as mean ± SD, n = 4; * = p < 0.05, compared to day 0

Hepatic MDR Increases as α-Tocopherol Decreases

* = p < 0.01, compared to day 0

Mustacich, et al.
Free Radic Biol Med
41, 1069-1078 (2006)

Hepatic α-Tocopherol Metabolism and Biliary Excretion

Nutrient/Drug Interaction between Vitamin E and HMG-CoA Reductase Inhibitors in Hypercholesterolemics

Objective
- To examine the nutrient-drug interaction with vitamin E and HMG-CoA reductase inhibitors (CYP3A4 enzyme substrates)

Study design
- Randomized, blinded, placebo-controlled trial

Hypothesis
- Vitamin E induces CYP 3A4 enzyme and thereby increases the clearance of the drugs that are metabolized by this enzyme
  - Lovastatin and Simavastatin are both metabolized by CYP 3A4
  - Since they lower serum cholesterol, increased drug metabolism should increase cholesterol
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Inclusion Criteria

- Diagnosis of hypercholesterolemia
- Lovastatin or simvastatin therapy
  - Dose had to be stable for at least 2 months at randomization
- BMI < 35 kg/m²
- Age > 18 y

Baseline Characteristics

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<tr>
<td>M/F (n)</td>
<td>11/10</td>
<td>12/11</td>
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<tr>
<td>Lova/Simva (n)</td>
<td>12/9</td>
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<tr>
<td>TC (mg/dl)</td>
<td>192 ± 7</td>
<td>182 ± 6</td>
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<tr>
<td>HDL (mg/dl)</td>
<td>57 ± 3</td>
<td>52 ± 3</td>
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<tr>
<td>LDL (mg/dl)</td>
<td>107 ± 5</td>
<td>102 ± 5</td>
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<tr>
<td>TG (mg/dl)</td>
<td>142 ± 18</td>
<td>140 ± 14</td>
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</tbody>
</table>

PLasma α-Tocopherol Increased in Response to 400 IU Vitamin E Supplements

- Placebo group:
  - wk 0 vs wk 4, 8, NS

- Vitamin E group:
  - wk 0 vs wk 4, p<0.001
  - wk 0 vs wk 8, p<0.001

No Change in Total Cholesterol

- Within group differences wk 0 to week 8, NS

No Change in LDL Cholesterol

- Within group differences wk 0 vs wk 8, NS

CYP3A4 Activity, Apparently Unchanged

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Summary of findings

- 8 weeks of 400 IU vitamin E supplements
- No significant effect seen on:
  - Total Cholesterol
  - LDL Cholesterol
  - CYP3A4
- Caveats
  - Limited number of patients studied

Vitamin E Revisited

- Mechanism of action
- The controversy: vitamin E's benefits in human health and chronic disease prevention
- Potential mechanisms for vitamin E's benefits

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