Vitamin E Revisited

Maret Traber, PhD

Vitamin E Revisited

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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

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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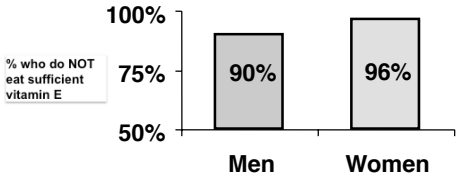
Food Sources of Vitamin E

Food	Serving	mg
Cereals ready-to-eat, fortified	1 cup	20.3
Almonds	1 oz (24 nuts)	7.3
Sunflower seeds, dry roasted	1/4 cup	6.8
Hazelnuts	1 oz	4.3
Oil, sunflower	1 tbsp	5.6
Oil, safflower	1 tbsp	4.6
Oil, canola	1 tbsp	2.4
Oil, olive	1 tbsp	1.9
Oil, soybean	1 tbsp	1.1
Spinach, cooked	1 cup	6.7
Tomato sauce	1 cup	5.1
Turnip greens, cooked	1 cup	4.4
Beet greens, cooked	1 cup	2.6
Potato chips	1 oz	2.6
Potato, french fried	1 large	2.6
Carrot juice	1 cup	2.7
Sweet potato, canned	1 cup	2.6
Broccoli, chopped, cooked	1 cup	2.4
Peppers, sweet, red, raw	1 cup	2.4

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



Moshfegh et al., What we eat in America, NHANES 2001-2002: usual nutrient intakes from food compared to dietary reference intakes USDA, Agricultural Research Service, 2005.

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

- 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

Wright et al., *Am J Clin Nutr* **84**, 1200-7 (2006).

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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
  - ➔ 115 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
  - ➔ (95 percent confidence interval, 0.38 to 0.91)
- Stampfer, MJ NEJM 328:1444, 1993

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JAMA Feb 28, 2007

Mortality in Randomized Trials of Antioxidant Supplements for Primary and Secondary Prevention

Systematic Review and Meta-analysis

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*

Vitamins E and C in the Prevention of Cardiovascular Disease in Men

The Physicians' Health Study II Randomized Controlled Trial

Howard D. Sesso, ScD, MPH
Julie E. Buring, ScD
William G. Christen, ScD
Tobias Kurth, MD, ScD
Charlene Belanger, MA

Context Basic research and observational studies suggest vitamin E or vitamin C may reduce the risk of cardiovascular disease. However, few long-term trials have evaluated men at initially low risk of cardiovascular disease, and no previous trial in men has examined vitamin C alone in the prevention of cardiovascular disease.

Objective To evaluate whether long-term vitamin E or vitamin C supplementation decreases the risk of major cardiovascular events among men.

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*

Vitamins E and C in the Prevention of Prostate and Total Cancer in Men

The Physicians' Health Study II Randomized Controlled Trial

J. Michael Gaziano, MD, MPH
Robert J. Glynn, ScD

Context Many individuals take vitamins in the hopes of preventing chronic diseases such as cancer, and vitamins E and C are among the most common individual supplements. A large-scale randomized trial suggested that vitamin E may reduce risk of pros-

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.

N SOME OBSERVATIONAL STUDIES, IN-

Intervention Individual supplements of 400 IU of vitamin E every other day and 500 mg of vitamin C daily.

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

Effect of Selenium and Vitamin E on Risk of Prostate Cancer and Other Cancers

The Selenium and Vitamin E Cancer Prevention Trial (SELECT)

Scott M. Lippman, MD
Eric A. Klein, MD
Phyllis J. Goodman, MS
M. Scott Lucia, MD

Context Secondary analyses of 2 randomized controlled trials and supportive epidemiologic and preclinical data indicated the potential of selenium and vitamin E for preventing prostate cancer.

Objective To determine whether selenium, vitamin E, or both could prevent pros-

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).

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Vitamin E in Thromboembolism Prevention—Women's Health Study

- 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 α-tocopherol's pharmacologic effects that result in decreased clot formation
- Glynn et al., Circulation 116, 1497-503, 2007

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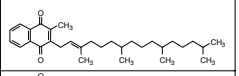
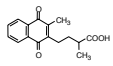
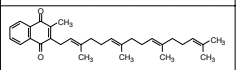
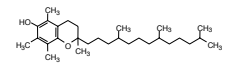
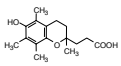
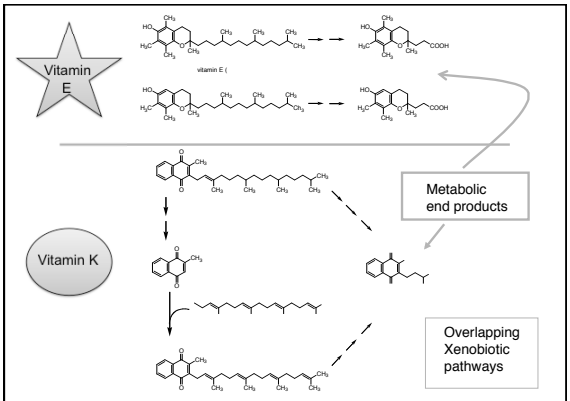
Vitamin E & K interactions

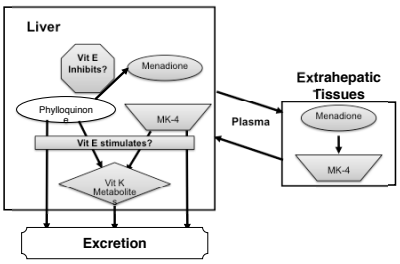
- Vitamin E is an antithrombotic agent, decreasing platelet aggregation
- Vitamin K is involved in the blood clotting cascade
- Vitamin E and K interactions have been recognized for >50 years, but the mechanisms for the interaction are still unknown
- Hypothesis: beneficial vitamin E effect in thrombosis could result from an interaction with vitamin K metabolism, decreasing the activation of vitamin K

Vitamin K and E structures

Vitamin		Metabolite
Phylloquinone (K1)		
Menaquinone (MK-4)		
α-tocopherol (E)		



Vitamin K activation



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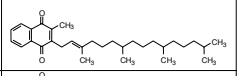
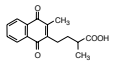
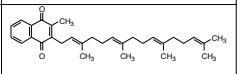
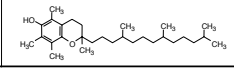
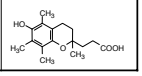
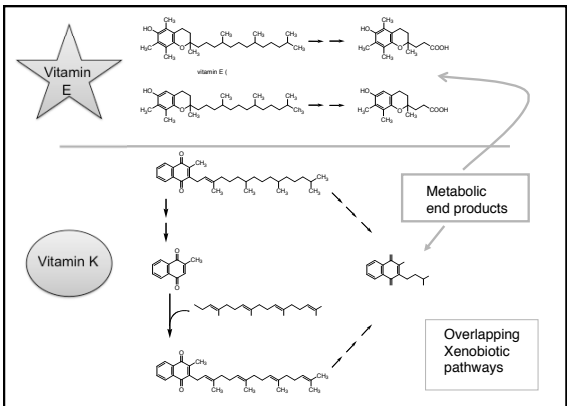
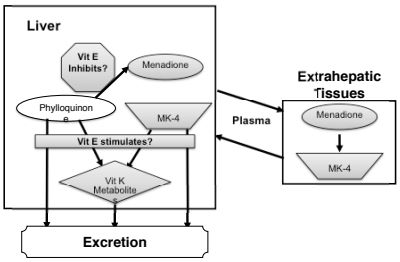
Phylloquinone (K1)		
Menaquinone (MK-4)		
α-tocopherol (E)		



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?

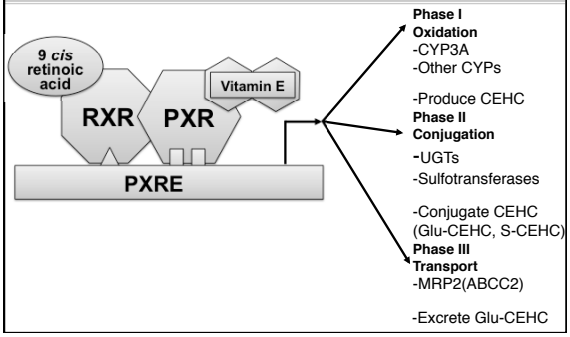
- Xenobiotic: chemical compound that is foreign to the body of a living organism.
  - ➔Drugs, pesticides, vitamins, herbs
  - ➔Any compound not synthesized by the body itself

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

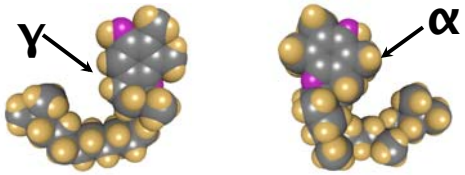
    (LeCluyse, '01, Lehmann et al, '98)
- CYP3A4 metabolizes more than 50% of drugs (Kliewer et al '02)

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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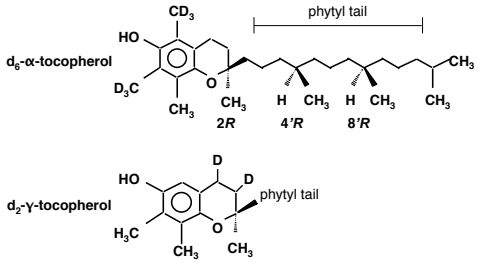
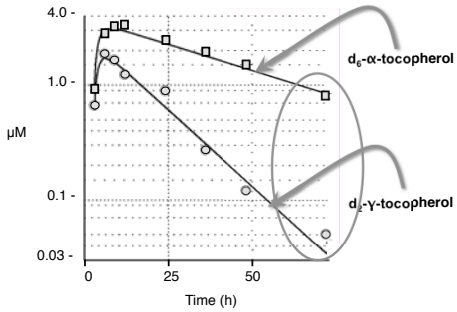
Deuterated α- and γ-Tocopherols



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

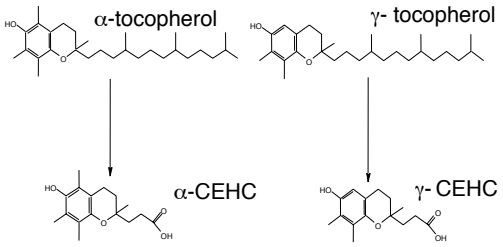


α-Tocopherol is more effectively retained in the plasma

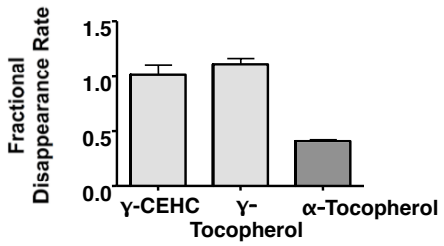
	Fractional Disappearance Rates (pools per day)	Half-Life (hours)
RRR-α-tocopherol	0.3 ± 0.1	57 ± 19
SRR-α-tocopherol	1.2 ± 0.6	16 ± 6
β–tocopherol	1.4 ± 0.4	13 ± 4
α-tocotrienol	4.0 ± 0.9	~4

Traber et al., *Proc. Natl. Acad. Sci. USA* **91**, 10005-10008 (1994).
Leonard et al., *Free Radic. Biol. Med* **38**, 857-66. (2005).
Yap et al., *J Pharm Pharmacol* **53**, 67-71 (2001).

α- and γ-Carboxy ethyl hydroxy chromanols and Their Respective Tocopherol Precursors



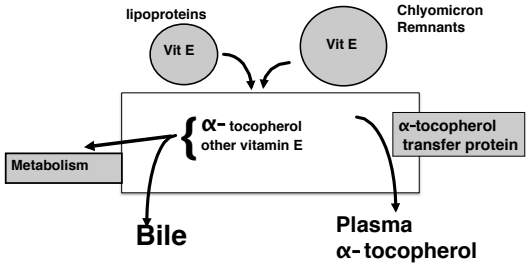
γ-Tocopherol and its metabolite disappear rapidly from plasma



Bruno et al., *Free Radic Biol Med* **40**, 689-97 (2006).

Regulation of Liver Vitamin E

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



Kayden, H.J. and M.G. Traber. J. Lipid Res. 34: 343-358, 1993.

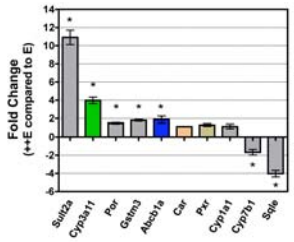
Altered gene expression in mice consuming 1000 IU vs 30 IU dl-α-tocopherol

Gene name	Gene ID	GenBank	Fold change	Direction
Sulfotransferase 2	Sult2a2	NM_009286	10.8	up
Multidrug resistance protein	Abcb1a	M30697	2.0	up
Glutathione transferase	Gstm3	J03953	1.9	up
Orphan nuclear receptor	mCar	AS009328	1.8	up
Pregnane X receptor	Pxr	AF031814	1.6	up
P450 oxidoreductase	Por	NM_008898	1.5	up
Cytochrome P450 3a11	Cyp3a11	BC010528	1.3	up
Cytochrome P450 1a1	Cyp1a1		NC	NC
Cytochrome P450 2b10	Cyp2b10		NC	NC
Cytochrome P450 2c	Cyp2c		NC	NC
Cytochrome P450 4f	Cyp4f		NC	NC
Squalene epoxidase	Sqle	NM_009270	2.7	down
Cytochrome P450 7b1	Cyp7b1	NM_007825	2.2	down

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-ΔΔCt
  - ➔Genes changed ≥ 1.5-fold with $P < 0.05$ were considered significant

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



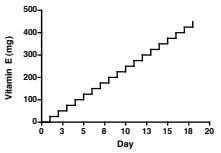
Xenobiotic Metabolism in Rats Modulated by Vitamin E Given as Subcutaneous Injections

Protocol

- Rats injected α-T daily s.c.
  - ➔ 10 mg /100g body wt., Vital-E 300, 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

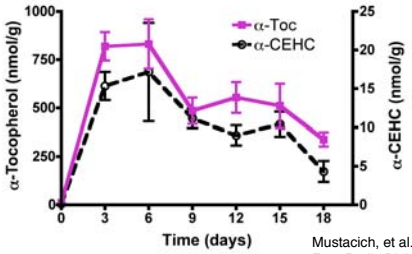
Mustacich, et al. Free Radic Biol Med 41, 1069-1078 (2006)

Cumulative vitamin E administered during the injection protocol



Total = 0.54 g/rat

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



mean ± SD, n = 4; all points $p < 0.05$ vs day 0

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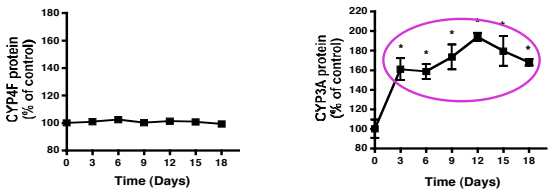
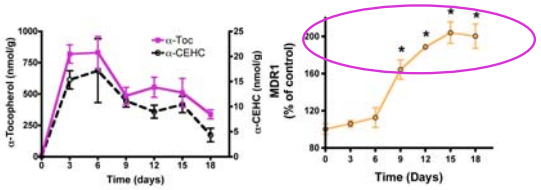
Hepatic CYP3A increases, while CYP4F protein levels remain unchanged



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

Hepatic MDR Increases as α-Tocopherol Decreases



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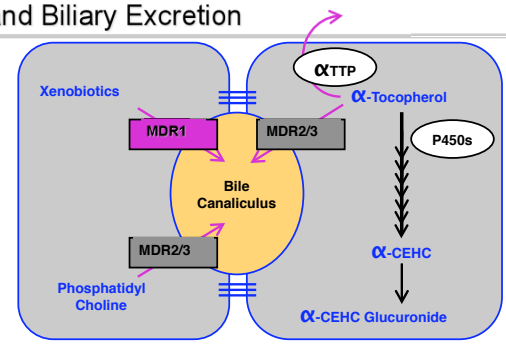
* = p < 0.01, compared to day 0

Hepatic α-Tocopherol Metabolism and Biliary Excretion



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

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

- 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

Vitamin E Revisited

Maret Traber, PhD

Inclusion Criteria

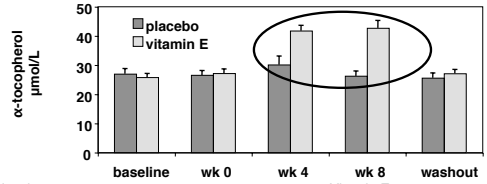
- Diagnosis of hypercholesterolemia
- Lovastatin or simvastatin therapy
  - ➔ Dose had to be stable for at least 2 months at randomization
- BMI < 35 kg/m^2
- Age > 18 y

Leonard, et al. Am J Health Syst Pharm 64, 2257-2266 (2007)

Baseline Characteristics

	Vitamin E N=21	Placebo N=23
Age (yrs)	66 ± 2.6	64.5 ± 8.5
M/F (n)	11/10	12/11
Lova/Simva (n)	12/9	15/8
TC (mg/dl)	192 ± 7	182 ± 6
HDL (mg/dl)	57 ± 3	52 ± 3
LDL (mg/dl)	107 ± 5	102 ± 5
TG (mg/dl)	142 ± 18	140 ± 14

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



Placebo group:

wk 0 vs wk 4, or 8, NS

Vitamin E group:

wk 0 vs wk 4, p<0.001

wk 0 vs wk 8, p<0.001

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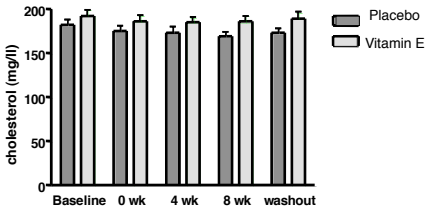
No Change in Total Cholesterol



Within group differences week 0 to week 8, NS

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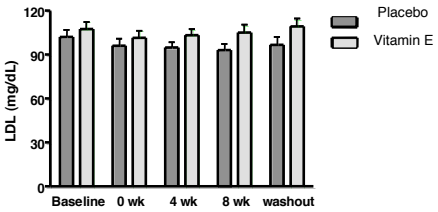
No Change in LDL Cholesterol



Within group differences wk 0 vs wk 8, NS

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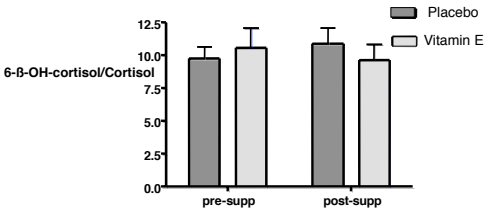
CYP3A4 Activity, Apparently Unchanged



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Vitamin E Revisited

Maret Traber, PhD

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

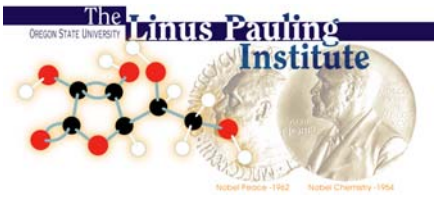
Leonard, et al. Am J Health Syst Pharm 64, 2257-2266 (2007)

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

Acknowledgements

- Traber Lab
  - ➔ Rich Bruno
  - ➔ Scott W. Leonard
  - ➔ Debbie Mustacich
- Brock University
  - ➔ Jeff Atkinson
- Columbia University
  - ➔ Rajasekhar Ramakrishnan
- UC Davis
  - ➔ Carroll Cross
  - ➔ Bettina Shock
  - ➔ Kishor Gohil
- Linus Pauling Institute
  - ➔ Dave Williams
  - ➔ Sharon Krueger
- Good Samaritan Regional Medical Center
  - ➔ Young-Sook Lee
  - ➔ David Blatt
  - ➔ Jacqueline Joss
- Natural Source Vitamin E Association for deuterated tocopherols and support of LC/MS purchase
- Supported by California TRDRP 7RT-0160, NIH ES11536 & DK59576



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