III. FAT SOLUBLE VITAMINS

A. Generalities

1. Are absorbed in association with lipids -- bile required for absorption; diseases that impair fat absorption can lead to deficiencies.

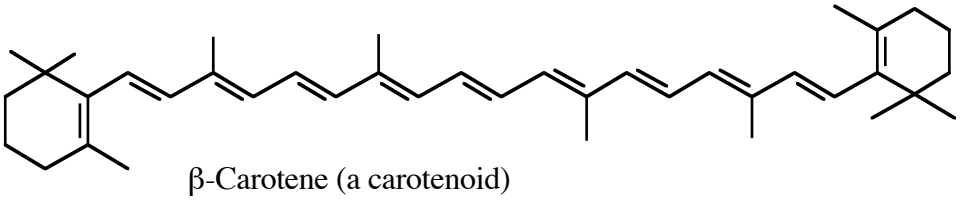
2. The fat soluble vitamins do not serve as coenzymes but rather act directly or bind to specific receptors in the cell nucleus to influence gene expression.

3. Vitamins A and D are stored in liver and it takes time to bring on a deficiency state.

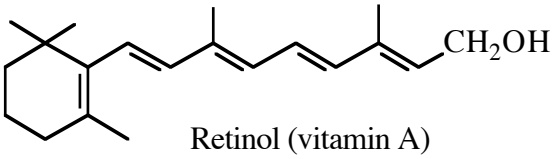
B. Vitamin A

1. <u>Chemistry</u>

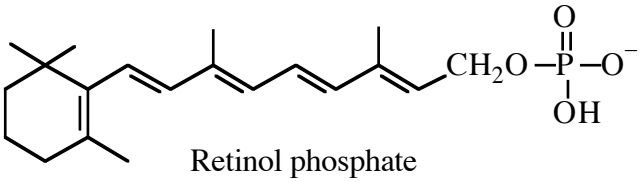
a. A series of compounds are active. Carotenoids are from plants, the retinoids from animals. Of the carotenoids, beta carotene is the most potent but some other plant carotenoids have provitamin A activity. Beta carotene is oxidized to yield (theoretically) 2 moles of retinal. Retinol (mixed esters, especially retinol palmitate) is mostly what is ingested from animal products.



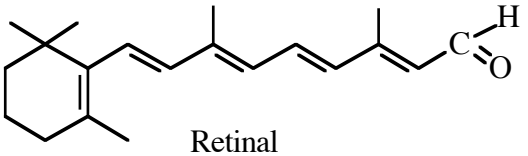
β-Carotene (a carotenoid)



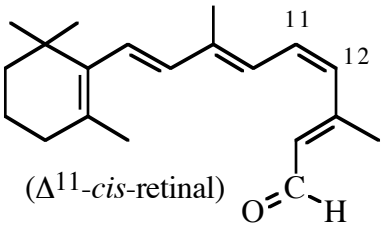
Retinol (vitamin A)



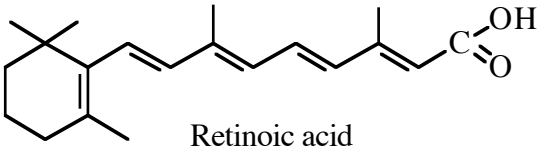
Retinol phosphate



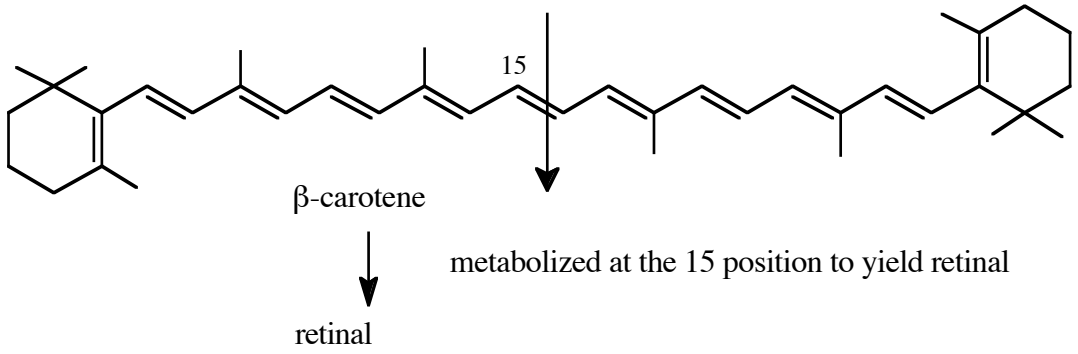
Retinal



(Δ^{11}-*cis*-retinal)



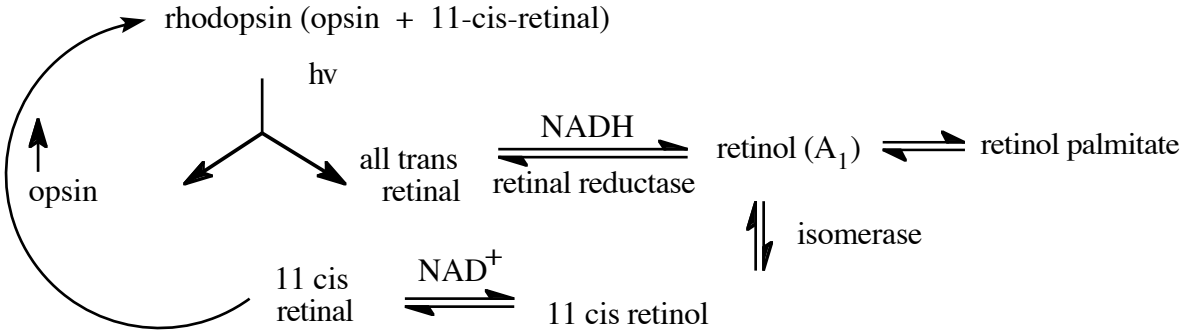
Retinoic acid



2. **Source** – plants provide carotenoids, animals provide retinol palmitate.

3. **Transport and storage** specific transport proteins exist for retinoic acid, retinal and retinol. Stored in liver as retinol esters (mostly retinol palmitate)

4. **Deficiency state** –
   a. Eye- night blindness. 11-cis-retinal binds to opsin to form the low light sensitive rhodopsin. See scheme below.



   b. Eye- keratinization of the cornea results in xerophthalmia and risk of blindness especially in children.

   c. Skin- much can be explained by the ability of retinol and retinoic acid to regulate macromolecule synthesis. In the skin, absence results in low mucin synthesis and high keratin synthesis (hyperkeratosis). Fissures allow microbe penetration and infection. Vitamin A is known as the "antiinfective vitamin".

   d. Immunity- a role of the vitamin in immune functioning especially in the activation of T-lymphocytes. Measles is a killer in kids with low vitamin A.

   e. Iron- vitamin A involved in synthesis of transferrin so that low erythrocyte iron results in anemia.

   f. Antioxidant/free radical scavenger- carotenoids have this activity

g. Cell differentiation: retinoic acid important and low vitamin A associated with an increased risk of various cancers.

h. Bone – low intake and high intake increases risks for weak bones.

6. **Requirement** -- adult DV = 5,000 I.U., RDA = 900 μg retinol equivalents (~ 2900 IU) for males and 700ug/d (~ 2300 IU) for females. 1 retinol equivalent = 1 μg retinol = 2 ug supplement beta carotene or 12 μg dietary β-carotene. 1000 ug retinol is about 3,300 IU; UL=3000ug = ~10,000 IU

7. **Dietary source** – animal products provide retinol esters, plants provide carotenoids. The richest sources are animal livers and fish oils. Eggs and fortified dairy products are also important.

8. **Stability** -- O_2 labile, as are most unsaturated fats.

9. **Use**

a. Deficiency state.

b. skin-

a. Acne -- topically as retinoic acid. Systemically as 13-cis retinoic acid (isotretinoin) Accutane® Roche. Attention: these retinoids are strong teratogens.
b. Psoriasis – etretinate (Tegison®); acitretin (Soriatane®)

c. Cancer -- Vitamin A deficiencies associated with increased sensitivity to carcinogens and increased tumor incidence but prospective studies with supplements have not shown consistent benefit. There is an association of low carotene intake and increased risk of lung cancer in smokers. However, supplementation of beta carotene to smokers gave an increased risk of lung cancer!

d. Low vitamin A intake is associated with more severe infectious diseases including HIV. The infectious process lowers vitamin A also. Retinol being evaluated by the WHO in some developing countries to decrease mortality in children due to measles and other infectious diseases. One study showed that severe measles in the USA was associated with low retinol levels.

e) Fat soluble free radical scavenger as carotenoids.

f) lycopene a carotenoid (diets rich in tomatoes) with no vitamin A activity seems to have benefit in preventing prostate cancer. Doses of 6-30mg used.

g) luteine a carotenoid (diets rich in broccoli, spinach, and kale) with no vitamin A activity seems to have benefit in preventing macular degeneration. Supplements may help prevent progression. Doses of 7-20mg used.

10. <u>Toxicity</u>

a. Hypercarotenosis -- eat too many carrots -- turn yellow, but no harm done.

b. Hypervitaminosis A -- characterized by hydrocephalus, vomiting, hypercalcemia and brittle bones, fatigue, malaise, joint pain, headaches, rough skin, swellings on the extremities, papilledema caused by increased production of spinal fluid (symptoms of brain tumor), hepatotoxicity.

Can be precipitated by chronic ingestion of 25,000 to 50,000 I.U./day
Note: cod liver oil has about 5,000 IU/5ml. Liver has about 30,000 IU/3 oz.

c. Teratogenic? - recent evidence says yes in doses > 10,000 I.U./d.

Note: Watch out for polar bear liver -- has 20,000 to 30,000 I.U./g.

d. Risk for fractures – intakes of retinol from all diet and supplements over about 10,000 IU is associated with increased risk for fractures in men and women. Avoid single supplements and excess dietary intake unless there is a compelling reason to do so. Carotenoids are OK.

11. Consumer Counseling and Advice

a) Avoid doses over 5000IU/d of retinol.
b) Avoid frequent eating of liver and routine use of cod liver oil
c) Avoid beta carotene as a single dietary supplement, especially for smokers. The amounts in multivitamins are usually low.
d) Veggies are the best way to get needed amounts of vitamin A because carotenoids are not stored

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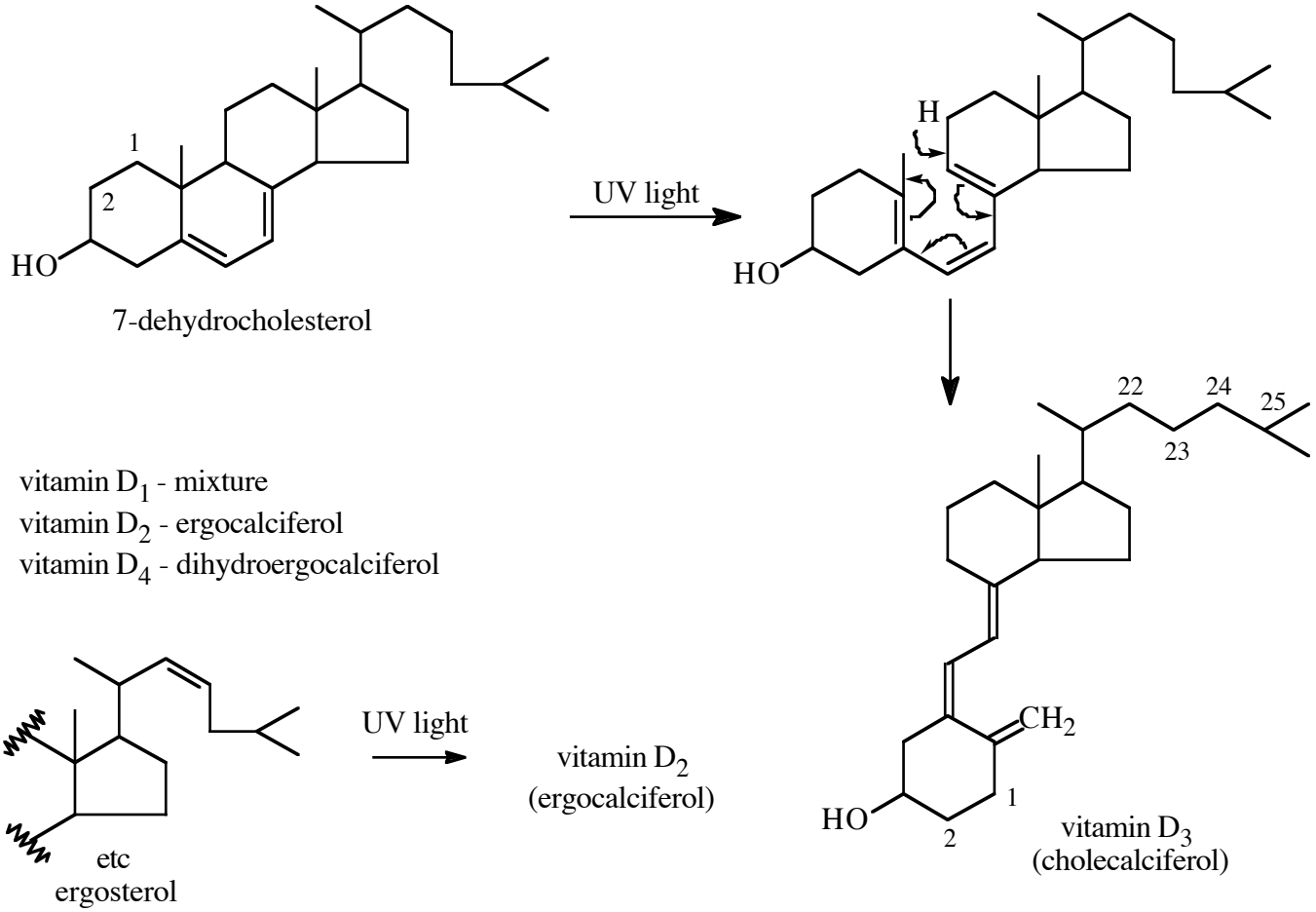
C. Vitamin D

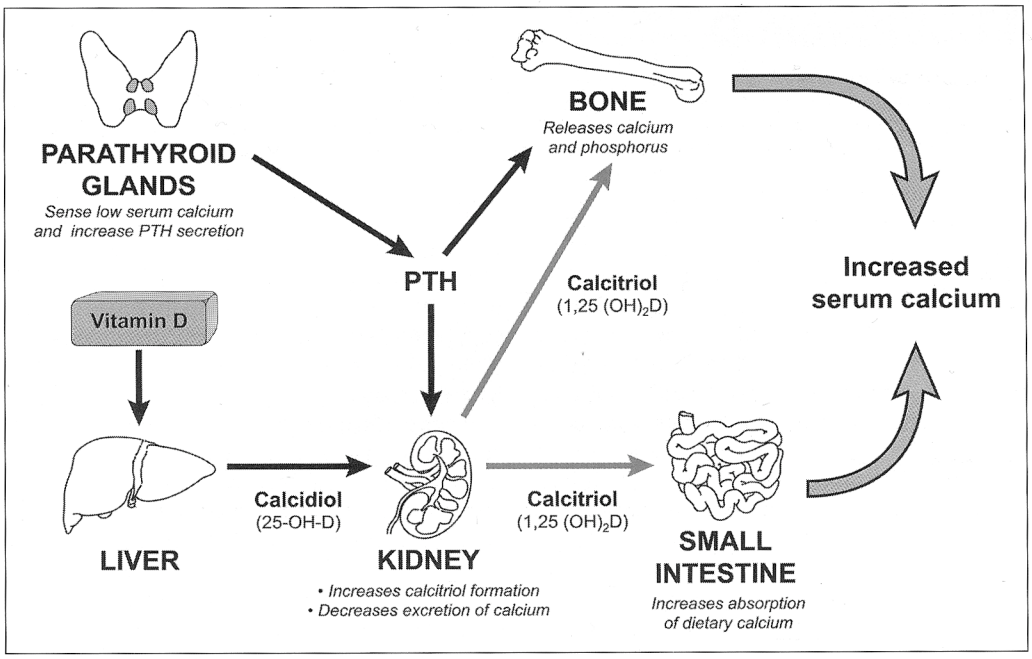
1. Structures D-1 through D-4



Note: D2 is not nearly as potent as D3 so watch labels on supplements

2. **<u>Deficiency state</u>** -- rickets, ostemalacia and maybe elevated risks for certain cancers and other diseases

3. **<u>Function</u>** -- Vitamin D as 1,25 DHCC is necessary for signaling gene transcription of calcium transporters that are involved in absorption of Ca through the intestinal mucosa. Vitamin D as 1,25 DHCC is also involved in cell regulation and differentiation.



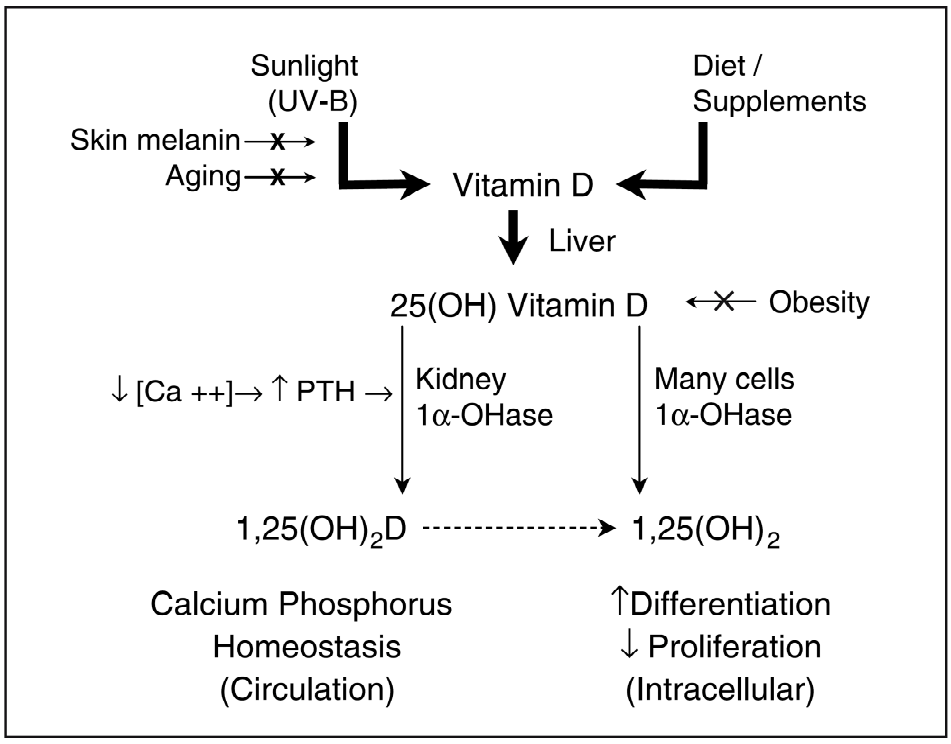
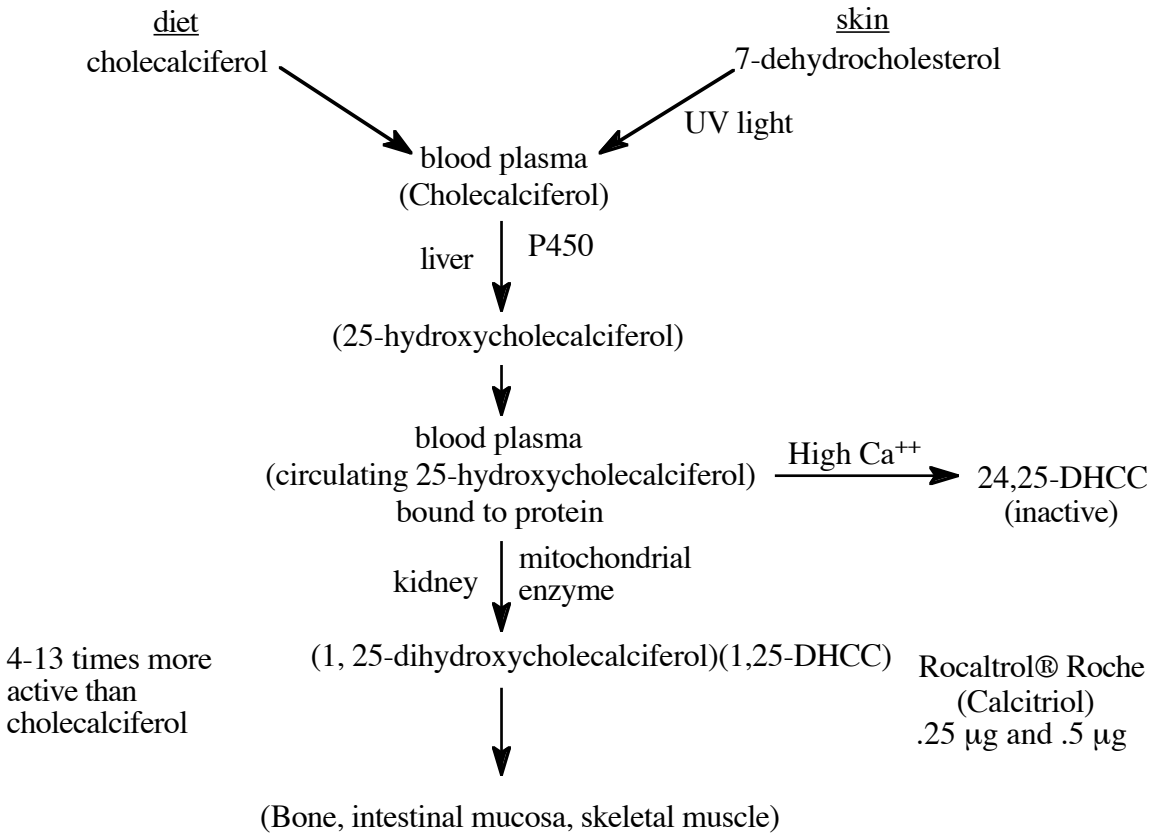


Fig 1. Proposed mechanism for vitamin D and cancer. The main sources of vitamin D are sunlight and diet or supplements. Darker skin, older age and obesity are associated with lower 25(OH)D. Anti-cancer effects may be largely due to conversion of 25(OH)D to $1,25(OH)_2D$ within cells, although circulating $1,25(OH)_2D$ may contribute.

4. **Metabolites** --



5. **Toxicity** -- avoid high doses, especially in infants; 400 units/day = potentially toxic for infant, 150,000 units/day = potentially toxic for adult, calcification of soft tissues (lung, kidney).

6. **Requirements** -- DV = 400 I.U.; AI = 15 μg (~600 IU) cholecalciferol. UL=2000 IU

7. **Source** -- fish liver, fish products, sunshine, eggs (in D supplemented hens), liver, milk (fortified). Cod liver oil has about 400 IU/5ml.

8. At risk for deficiency

i. Infants kept out of sun
ii. Elderly with minimal sun
iii. Dark skin with minimal sun
iv. Religions that cover the whole body.
v. Fat malabsorption
vi. Inflammatory bowel diseases
vii. Kidney failure
viii. Seizure disorders treated with anticonvulsants which increase D elimination

9. **Use** – the importance of adequate intake and perhaps the value of supplements of this vitamin are now beginning to be realized

- Deficiencies due to low sun exposure
- hypoparathyroidism to keep calcium serum levels adequate
- osteomalacia and osteoporosis.
- Use 1,25 DHCC in renal failure.
- There is now strong evidence that vitamin D supplements and calcium help prevent fractures in postmenopausal women. (20-30% decrease). Most studies used 700-900 IU per day.
- There is strong interest in a potential role of vitamin D and calcitriol in the prevention of several cancers, especially colon, breast and prostate. There is an inverse association of D & Ca intake and risk for these cancers.
- There is an overall small association of vitamin D supplement use and decreased death due to all causes.
- MS risk. Seems to be an inverse relationship of intake and risk.

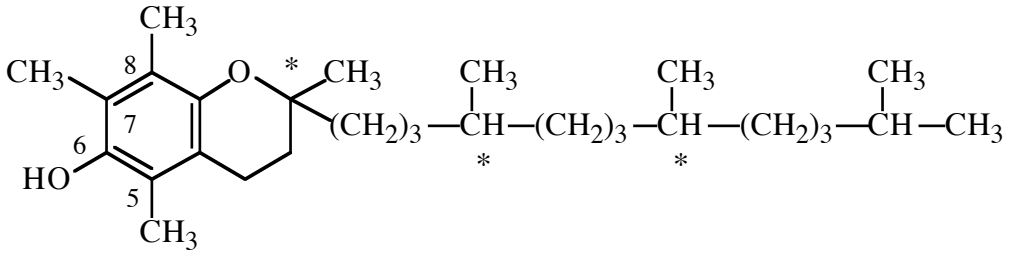
Calcitriol (Rocaltrol® Roche and generic products) -- 1,25-dihydroxy D_3 -- the "active" metabolite. Available in capsules and injection.
Paricalcitol (Zemplar®)–
Modification of 1,25 DHCC used PO for hyperparathyroidism associated with renal failure. 1ug 3 times per week.

9. Consumer Counseling and Advice

- **a.** Assure intake of at least 400IU/d. Multivitamins usually contain this amount.
- **b.** There is evidence that more than 400IU/d may be beneficial if sun exposure is minimal. 800IU/d seems optimal based on evidence today.
- **c.** Look to make sure intake is vitamin D3 not D2. D2 does not elevate needed vitamin D metabolites as does D3.
- **d.** Vitamin D is very important for bone health but also may help reduce risks for cancer and other diseases.
- **e.** Postmenopausal women should take a vitamin D supplement as well as Ca

D. Vitamin E

1. Structure



There are 8 possible stereoisomers. "Natural" vitamin E is RRR-α-tocopherol. RBC levels of RRR are higher than with an equal dose of the racemate.

2. **Activity**

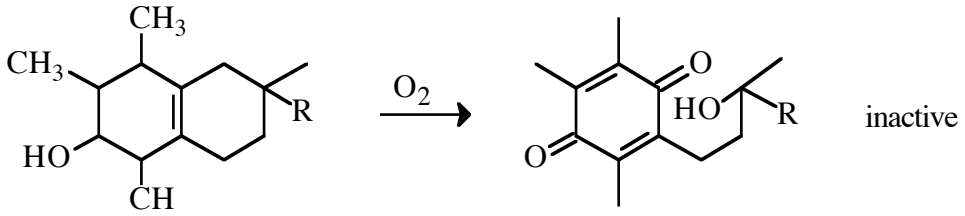
d-α-tocopherol	1 mg = 1.5 I.U.	
dl-α-tocopherol	1 mg = 1.1 I.U.	synthetic
dl-α-tocopherol acetate	1 mg = 1.0 I.U.	synthetic

Other tocopherols are active:

β = 7-demethyl	1 mg = 0.1 I.U.
α = 5-demethyl	1 mg = 0.1 I.U.
δ = 5,7-demethyl	1 mg = 0.1 I.U. (present in large amounts of corn)

3. <u>Stability</u>

Much is lost during processing and cooking. White, bleached flour has almost all E destroyed.

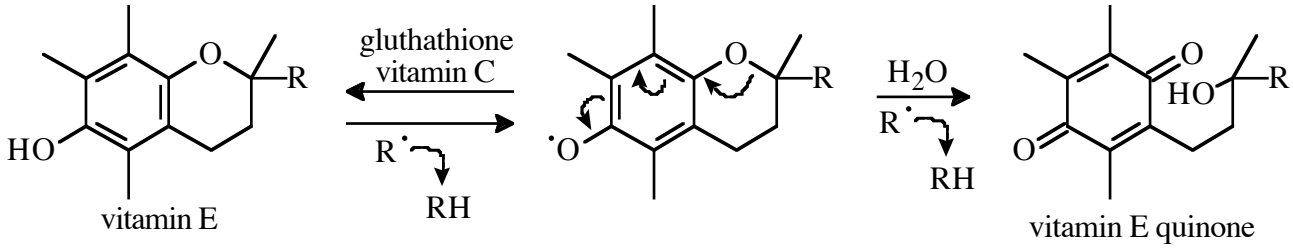


α tocopherylquinone

Iron catalyzes the oxidation.

3. <u>Properties</u> -- Good fat soluble, antioxidant, good chain breaking free radical scavenger.

Vitamin E as a free radical scavenger



4. <u>Effects</u>—

a. Has mild effect in decreasing platelet aggregation due to an effect on decreasing the activity of cyclooxygenase (therefore decreased conversion of arachadonic acid to thromboxane).
b. Decreases LDL oxidation
c. Decrease smooth muscle proliferation
d. Preserves endothelial cell function
e. Decreases monocyte reactive oxygen species (ROS)
f. Effects transcriptional regulation of some genes

5. <u>Distribution</u> -- Almost ubiquitous; rich sources are seed germ oils, green vegetables, whole grain cereals; margarine supplies much of our intake in the U.S.

6. Deficiency state

Animal Species	Syndrome
Rat (male)	sterility, liver necrosis
Rat (female)	fetal resorption, liver
Rabbit	muscular dystrophy, myocardial degeneration
Dog and Guinea Pig	Myocardial degeneration
Primate	Macrocytic anemia, muscular dystrophy

Man

A dietary deficiency of E is rare. A deficiency state was seen in some premature infants (stores of E are low at birth due to poor placental transport where edema and hemolytic anemia has been observed when the infants were fed a formula low in E and high in polyunsaturated fatty acid). In diseases resulting in malabsorption of fats, a neurological impairment observed has responded to vitamin E.

7. Requirement -- DV = 30 I.U. new RDA is 15mg (~22 IU of natural or 33 IU of synthetic). UL = 1000mg (~1500 IU natural or ~2200 of synthetic)

8. Biological function of E -- Acts as a general fat soluble biological antioxidant to prevent free radical oxidation of lipids and hence essential membrane destruction. More on this later.

9. Uses -- The claims for benefit of supplements of vitamin E are numerous and include increased virility, increased athletic performance, and help for diabetes, heart disease, dementia, cancer and aging.

f. Heart Disease--Retrospective studies have showed that daily use of vitamin E supplements in doses of > 100 IU have been associated with a decreased risk for coronary heart disease in both men and women (*New Eng. J. Med.* **328**:1450, 1993 and **328**:1444, 1993). However, dietary intake studies and retrospective studies may be confounded. Prospective studies in humans have not confirmed this and some have shown a slight increase in adverse outcomes with doses over 400IU/d. Many of the studies, however, have been secondary prevention studies in populations already at high risk.

g. Intermittent claudication -- Long-term treatment with 400 I.U./day; vitamin E has been reported to be of benefit in two older clinical studies.

h. Parkinson's disease – inverse risk relationship with increased dietary intake; observational studies

i. All cause mortality – A small **increase** at doses over 400IU. Most studies were in high risk populations, however. A small decrease in mortality in doses <400 IU (Ann Intern Med. 2005 Jan 4;142(1):I40.)

j. Tardive dyskinesia – some promise for benefit of supplements Ann Clin Psychiatry. 1998 Sep;10(3):101-5.

k. Pre-eclampsia – some promise in prevention with supplements

l. Athletic performance -- no benefit in competitive swimmers.

m. Nocturnal leg cramps -- Evidence is suggestive of benefit, but careful evaluation has yet to be done.

n. Increased sexual performance and function -- Sorry, no help here!

o. Retrolental fibroplasia and Brochopulmonary dyspasia. Eye and lung damage in premature infants on oxygen; as an antioxidant, E seems to offer some protection, but deaths have occurred. These deaths occurred in Wash. State and were associated with IV admin. of tocopherol. This form is no longer available and MVI Pediatric (Astra) is used as a source of IV Vitamin E for premature infants.

p. Dysmenorrhea – 2 studies showed benefit in teens.

q. Alzheimers Disease -- high doses (2000 IU/d) showed some benefit in decreasing progression but not in prevention.

r. Dementia – in >73 years old study population, long term use associated with decreased risk (but not for Alzheimer's)

s. Cancer – supplement use gave decreased risk for prostate cancer in smokers.

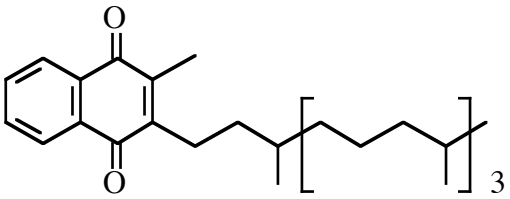
10. **Toxicity** --Tocopherols are generally considered non-toxic. Various rare diverse adverse effects have been reported. Exacerbated bleeding when given together with warfarin is the most significant drug interaction involving Vitamin E. Doses over the UL have this risk.

11. **Consumer Counseling and Advice**

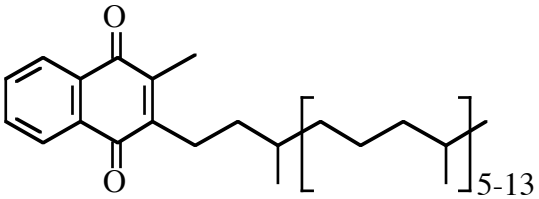
a) Based on recent evidence, supplements higher than 200IU are not beneficial and could be harmful
b) The amount in a multivitamin is probably adequate (30IU) for most
c) Natural vitamin E (RRR) is better utilized than the racemic synthetic
d) The hoped for benefit of high dose vitamin E supplements does not seem to have materialized.

E. Vitamin K -- Group of napthoquinones having antihemorrhagic activity.

1. <u>Chemistry</u>

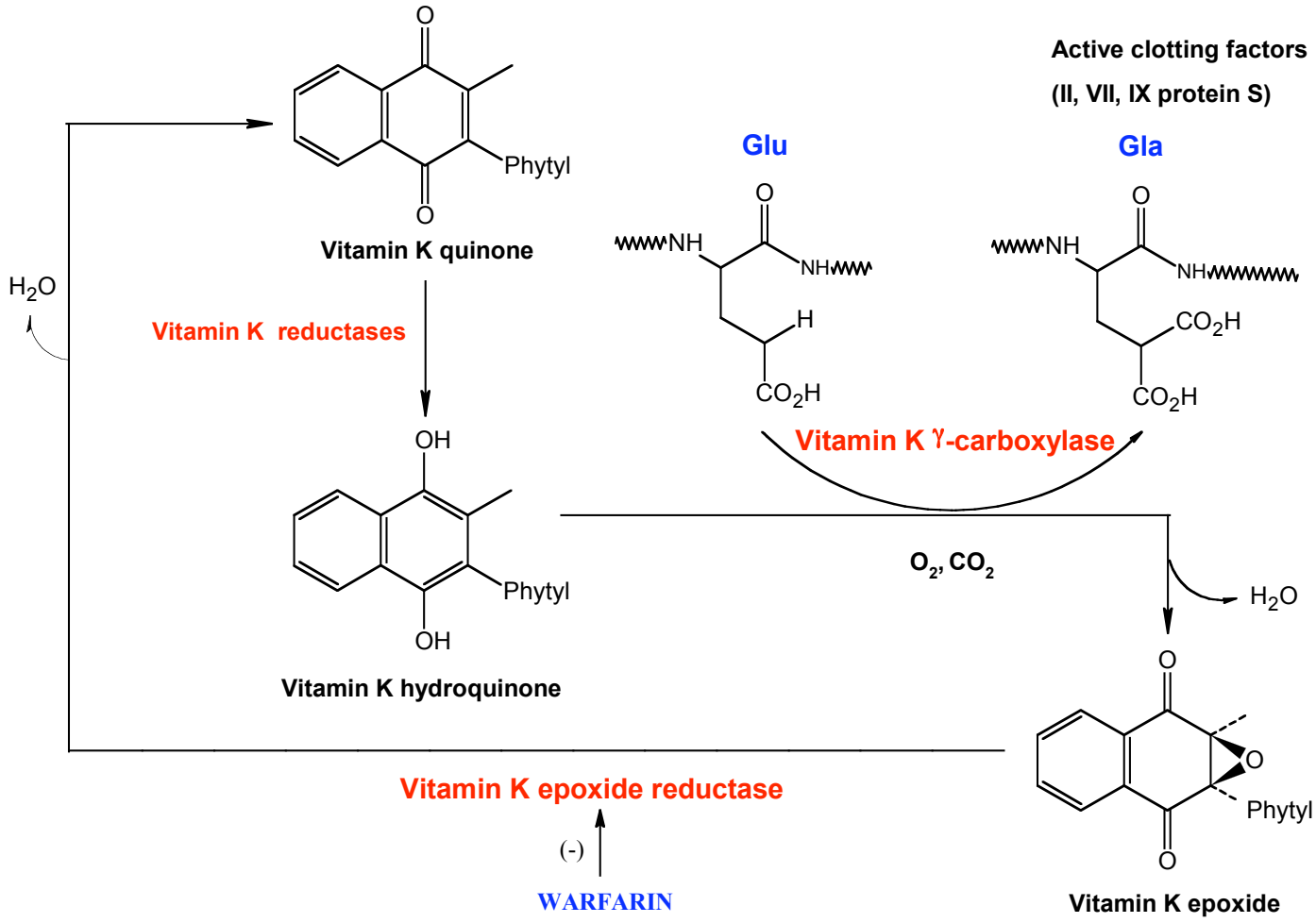


phytonadione (vitamin K_1)
(plants)



menaquinones
(from bacteria)

2. <u>Function</u> -- Necessary for carboxylation of γ glutamyl residues on precursor proteins → activation → blood coagulation.
Also γ carboxylation of osteocalcin in bone.



Vitamin K scheme for γ carboxylation of glutamyl residues on precursor clotting proteins

3. **Deficiency** -- Deficiency state can come on fast because K is not stored. Long term antibiotic therapy may increase risk of deficiency because ~ 50% comes from intestinal bacteria.

4. **Use** –
   g. Coagulation - Water soluble derivatives used for IV and where absorption is impaired. For an anticoagulant overdose, use K1. K1 is used routinely at birth IM to prevent neonatal hemorrhage.
   h. Bone – Gamma carboxylation of osteocalcin is enhanced with improved bone deposition. Use of 25mg/d for 2 years decreased hip fractures in an older population.

5. **Source** -- Spinach, cabbage, tomatoes, other green vegetables.

6. **Dose** – 80ug is DV. This may be too low for optimal activities. No UL.

7. **Toxicity**- some allergic reactions IV but not IM or PO. Otherwise nothing special.

8. Consumer Counseling and Advice

i. Adequate intake is important for the ability of blood to clot and for healthy bones
j. A good diet with leafy vegetables (and a healthy gut flora) can probably supply needs but the amount in most multivitamins will assure a good intake.
k. If on warfarin, then working with health care providers is important to adjust the warfarin dose and to keep vitamin K levels steady.

IV. OXIDATIVE STRESS AND PROTECTIVE MECHANISMS INVOLVING VITAMINS

Reactive oxygen species that result in tissue damage (drugs, pesticides, pollutants can cause tissue damage via oxidative metabolism).

Importance - inflammation, carcinogenesis, hemolysis, athrosclerosis, arthritis, aging, drug adverse effects.

Reactive O_2 species causing damage:

- Complete reduction of O_2 $\quad O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$

  but this goes in 1 electron steps

$$O_2 \xrightarrow{e^-} O_2^{\cdot -} \xrightarrow{e^-} O_2^{-2} \xrightarrow{e^-} \left[O^{-2} + O^{\cdot}\right] \xrightarrow{e^-} 2O^{-2} \xrightarrow{4H^+} 2H_2O$$

$$O_2^{-2} \xrightarrow{H^+} H_2O_2$$

- Reactive species

$$O_2^{\cdot -} + HOOH \xrightarrow[Fe^{++} \rightarrow Fe^{+++}]{Cu^{++} \rightarrow Cu^{+++}} OH^- + OH^{\cdot} + O_2$$

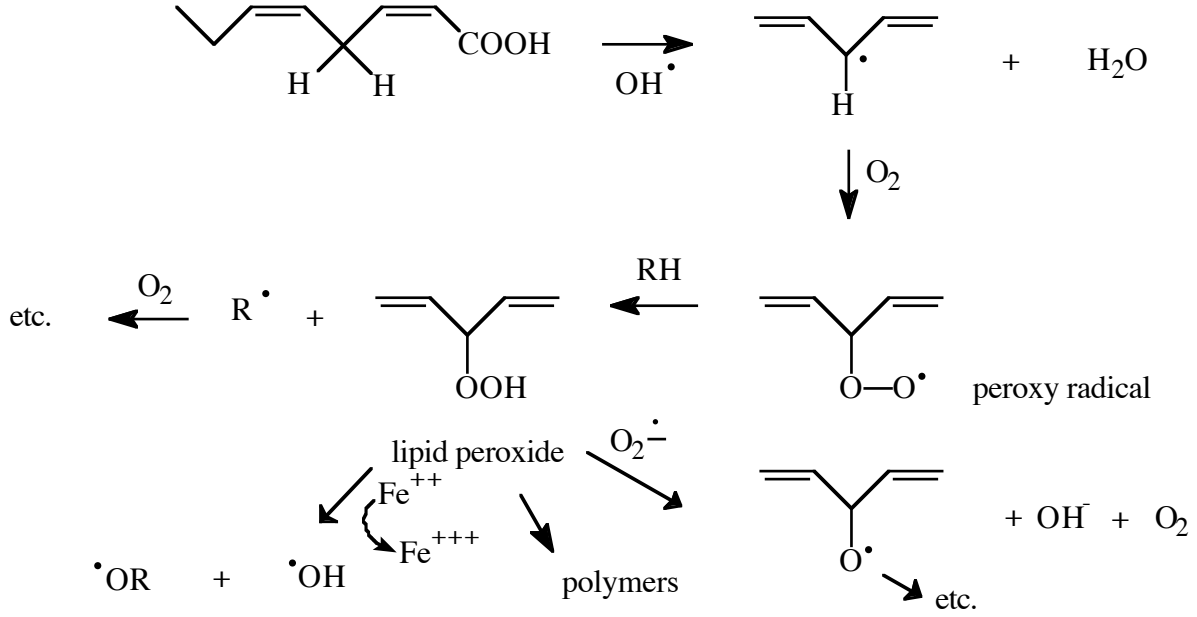
hydroxy anion; hydroxy radical

$$O_2^{\cdot -} + H^+ \longrightarrow HO_2^{\cdot}$$

perhydroxy radical

- Origin of e^- - oxidation of hydroquinones, flavins, thiols, drugs, mitochondrial respiration, microsomal reactions, UV light radiation, etc.

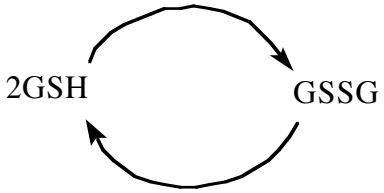
Targets - DNA, thiols, enzymes, membranes, collagen, lipids, e.g., unsaturated lipid.



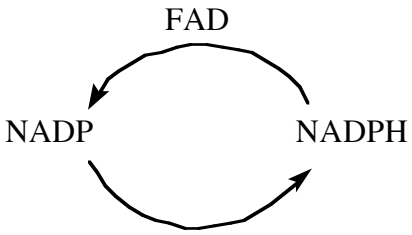
Protective Mechanisms

1. Glutathione pathway

Glutathione peroxidase (Selenium)



Glutathione reductase (FAD)



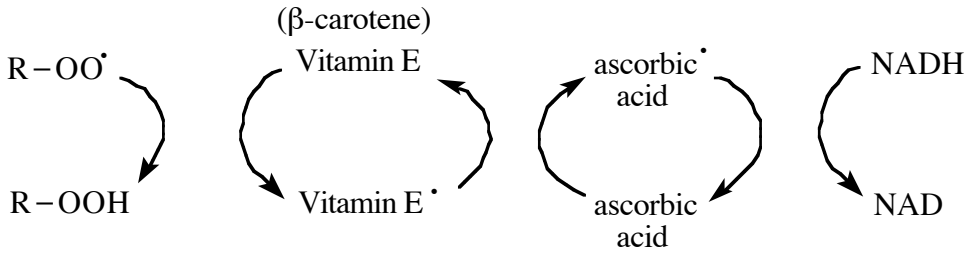
Glucose –6–phosphate dehydrogenase

Glucose –6–phosphate 6–phosphogluconic acid

2. Superoxide dismutase: $2\ O_2^{-\cdot} \xrightarrow{H^+} H_2O_2 + O_2$

3. Catalase: $2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$

4. Vitamins A, β-carotene, C, and E and selenium



Issues:

Should supplements of antioxidant vitamins be routinely recommended?
Is there evidence that antioxidant use has long term benefit?
Are there adverse consequences of taking antioxidant supplements?
What doses should be used if use is deemed safe and worthwhile?

In my opinion, the definitive answers to these questions are not yet available.

V. MULTIVITAMINS

A. Need?

Do we need to supplement diets with multivitamins? Many say no; others say maybe in certain circumstances, some say yes.

Cases where multivitamin supplements are clearly worthwhile:

Inadequate intake -- alcoholics, poor, elderly, dieters, poor diet

Poor absorption -- elderly, GI disorders, cystic fibrosis, diarrhea

Increased needs -- pregnancy, lactation, infants, smokers, injury, trauma, surgery, infection

Iatrogenic Vitamin Deficiencies -- oral contraceptives, long term antibiotic use, isoniazid, cholestyramine.

B. Available multivitamin preparations -- product selection guidelines