

Allan Rettie, [rettie@uw.edu](mailto:rettie@uw.edu)

Office – H164B; 206-543-0615

## Fat-Soluble Vitamins: History and General Properties

- ADEK is the acronym, 'fat cat is in the attic' (world's worst mnemonic).
- Early work on vitamins A and D because of their role in children's diseases.
  - ✓ Xerophthalmia (blindness)
  - ✓ Rickets
- Second phase of interest in vitamin D in the 1980s when the 'vitamin D cancer hypothesis' emerged.
- Unlike water-soluble vitamins that need regular replacement in the body, typically fat-soluble vitamins are stored in fatty tissues; e.g. adipose tissue, skeletal muscle, liver. Consequently, it takes time to bring on a deficiency state (rare in US with an adequate diet). However, they are more likely to cause toxicity on over-dosage (e.g. mega-doses of vitamin supplements).
- Unlike water-soluble vitamins, notably the B-family that serve as coenzymes/cofactors for energy-producing reactions, the fat-soluble vitamins either act directly (e.g. vitamin E), bind to specific receptors in the cell nucleus to influence gene expression (e.g. vitamin D, vitamin A) or act as cofactor for Gla protein production (vitamin K).
- Fat-soluble vitamins are absorbed, through the lymphatic system, in association with dietary fat - bile is required. Bile salts, free fatty acids and  $\beta$ -monoglycerides combine in mixed micelles, whose hydrophobic cores provide a suitable environment for the fat-soluble vitamins.
- Diseases that impair fat absorption such as ulcerative colitis, Crohn's) can lead to deficiencies, as can certain medications.
  - ✓ Cholestyramine
  - ✓ Mineral oil
- Fat-soluble vitamins are largely stable to heat/cooking. Several are light sensitive - vitamin A/vitamins E and K.

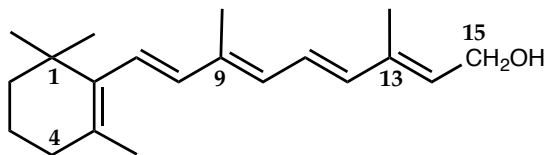
# VITAMIN A

## 1. Background

- Vitamin A – one of the first vitamins discovered - is a nutrient of global importance because shortages in its consumption are estimated to affect 200 million children worldwide and 20 million women of childbearing age, mainly in developing countries, especially S. Asia.
- Chronic deficiencies of vitamin A in susceptible populations contribute to child mortality secondary to infection and to the eye-diseases, xerophthalmia and night blindness.

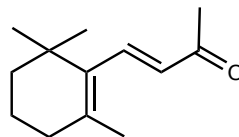
## 2. Chemistry and Metabolism

- Vitamin A is a generic descriptor for a series of **retinoid** and **carotenoid** compounds that exhibit the qualitative biological activity of retinol.

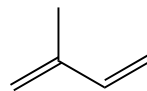


### Essential structural features of naturally occurring [preformed] vitamin A (retinol, retinal, retinoic acid)

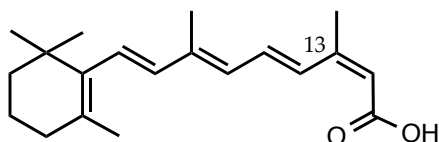
- Substituted  $\beta$ -ionone nucleus.



- Polar functional group at C-15 - alcohol, aldehyde or acid.
- Esterification occurs at C-15. Mixed esters, especially retinyl palmitate are the principal storage form and what is mostly ingested from animal sources.
- Side-chain at C-6 composed of isoprenoid units.



- Conjugated double-bond system extending from C-5. Retinol  $\lambda_{\max} = 325\text{nm}$ , retinoic acid  $\lambda_{\max} = 350\text{nm}$ .
- Many stereoisomers (*cis/trans*) possible, but most of the *cis* isomers are sterically hindered. Important exceptions are 11-*cis* retinal, and 9-*cis* and 13-*cis* retinoic acid.
  - 11-*cis* Retinal and 11-*trans* retinol undergo *cis-trans* isomerization, important in the visual cycle, catalyzed by light and certain metabolic enzymes, respectively.
  - *Trans* and *cis* isomers, e.g. 9-*cis* and 13-*cis* RA, are pharmacologically active and used in the treatment of cancer/acne.



## Carotenoids: Pro-Vitamins A

- Some compounds of the **Carotenoid** class of polyisoprenoid plant pigments (with absorbance at 400-500 nm) yield vitamin A upon metabolism and so are referred to as pro-vitamins A.
- Of the ~600 plant carotenoids identified, only the few that contain an unmodified  $\beta$ -ionone ring have pro-vitamin A activity. All have some antioxidant activity.
- $\beta$ -Carotene is the most potent carotenoid because it yields (theoretically) 2 moles of retinal after cleavage of the 15-15' C-C bond by  $\beta$ -carotene monooxygenase (BCMO) in the intestine. However, the enzymatic process is inefficient and conversion decreases when body stores of vitamin A are high.

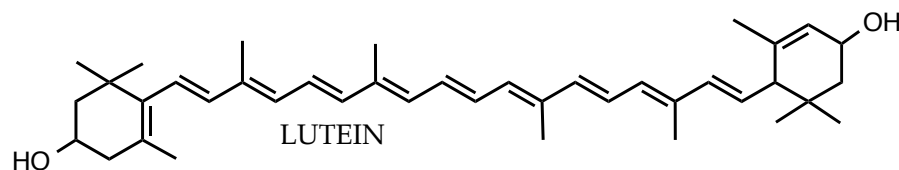
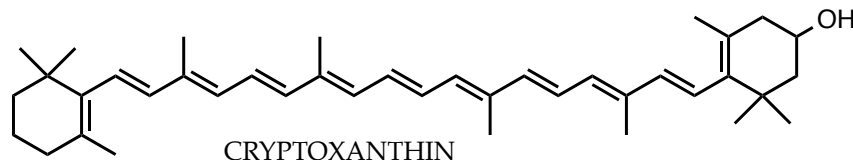
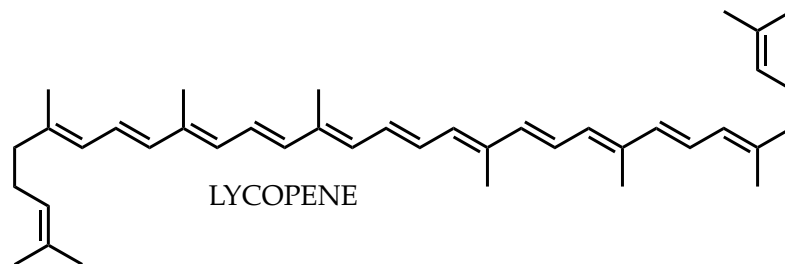
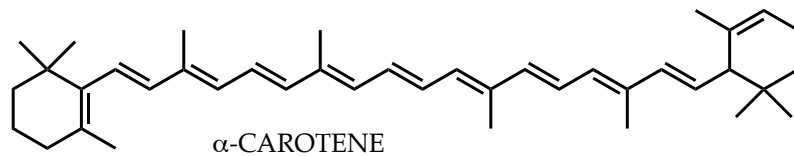
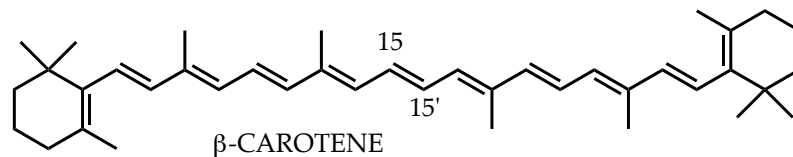
## CAROTENOIDS

### Carotenes

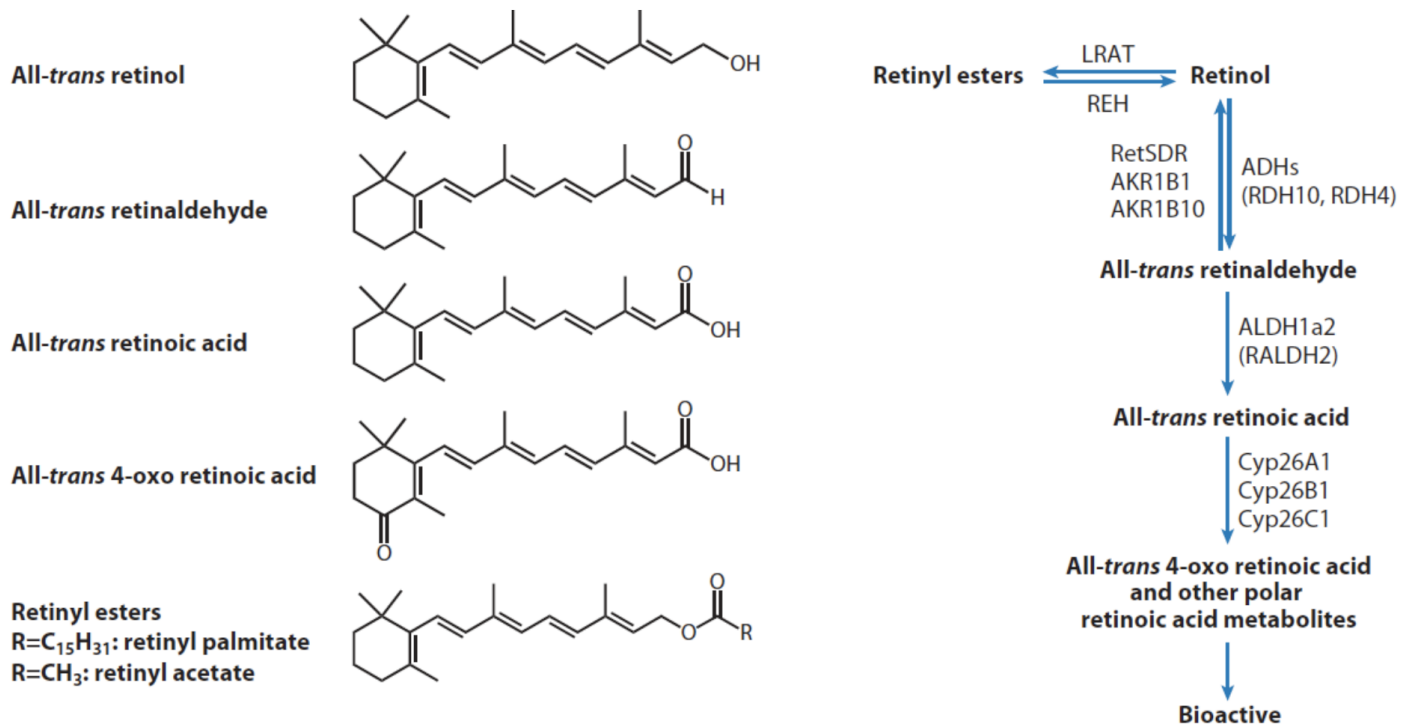
- $\beta$ -carotene
- $\alpha$ -carotene
- lycopene

### Xanthophylls

- cryptoxanthin
- lutein



## Metabolism



- Retinyl ester hydrolase (REH) hydrolyzes the esterified storage forms to liberate retinol.
- NAD-dependent Alcohol Dehydrogenases (RDH, RetSDR) and Aldo-Keto Reductases (AKR) convert retinol  $\leftrightarrow$  retinal, in a reversible manner.
- NAD(P)<sup>+</sup>-dependent Aldehyde Dehydrogenases (e.g. RALDH2) convert retinal  $\rightarrow$  retinoic acid in a multi-step process.
- The CYP26 enzymes, CYP26A1 and CYP26B1, metabolize retinoic acid by 4-hydroxylation on the  $\beta$ -ionone ring. The biological activity of P450 metabolites is under debate.

### 3. Dietary sources

- Plants (carrots, sweet potatoes, red peppers) provide carotenoids.
- Animal (livers) provide retinol esters (palmitate/stearate).
- Fish (liver) oils, eggs and fortified dairy products are also important sources of vitamin A.

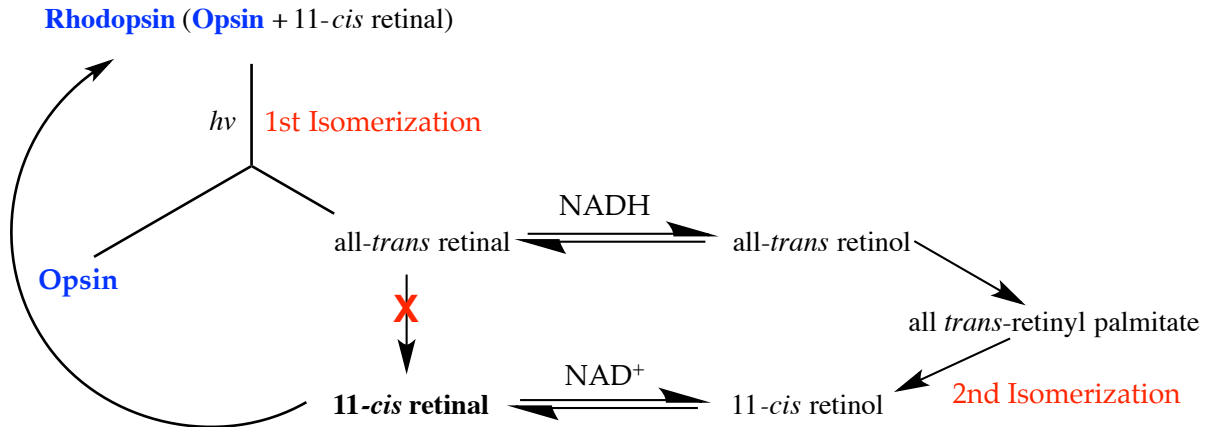
### 4. Transport and storage

- Specific transport proteins (CRBP, CRABP, RBP) exist for retinoic acid, retinal and retinol.
- Stored in liver as retinol esters (mostly retinyl palmitate).

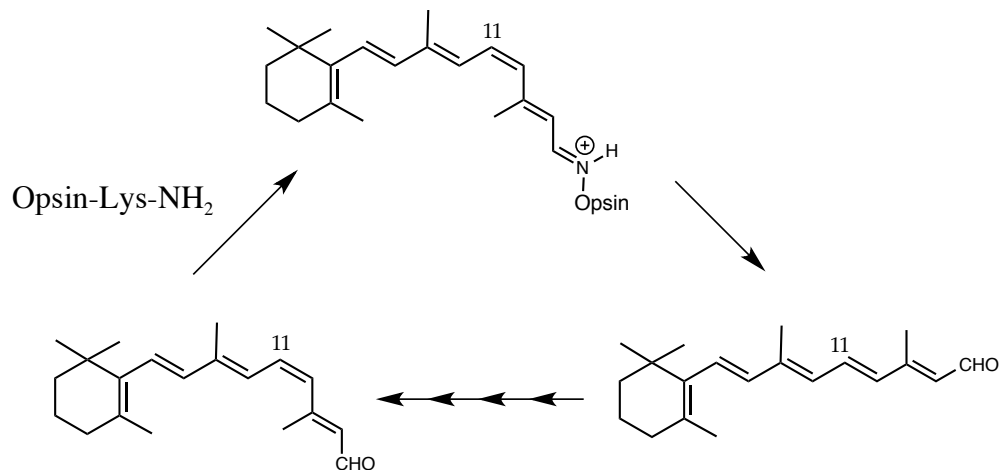
5. **Functions:** The active forms of vitamin A have 3 basic functions; *Vision, Growth and Development and Immunity.*

Vision – vitamin A is an integral part of the rhodopsin (present in rods) visual cycle.

- The rhodopsin cycle involves two critical isomerizations; the first, catalyzed by light, converts 11-*cis* retinal (bound to opsin) to all-*trans* retinal.

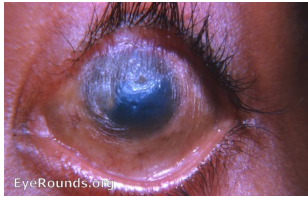


- The second, catalyzed by retinoid isomerohydrolase, converts *all trans* retinyl palmitate to 11-*cis* retinol.
- Oxidation of 11-*cis* retinol generates 11-*cis* retinal, which binds reversibly to opsin *via* a Schiff base with Lys296 (imine formation) to generate the low light sensitive pigment, rhodopsin.



## Growth and development

- Much can be explained by the ability of vitamin A to regulate cell synthesis of macromolecules (*via* RAR and RXR receptor activated transcription).
- Keratinization of the cornea results in *xerophthalmia* and risk of blindness, especially in children.



- Anemia - vitamin A is involved in synthesis of transferrin; deficiency causes low erythrocyte iron that results in anemia.
- Bone - Low intake, but especially high intake, increases risk for weak bones.
- Skin - Deficiency here results in low mucin synthesis and high keratin synthesis (hyperkeratosis). Fissures allow microbe penetration and infection. Vitamin A is known as the “anti-infective vitamin”.

## Immunity

- Deficiency causes widespread alterations in immunity, including pathological alterations in mucosal surfaces, impaired antibody responses to challenge with protein antigens, changes in lymphocyte subpopulations and altered T-cell and B-cell function.
- Measles is a killer in children with low vitamin A.

### 6. **Deficiency** – characterized by plasma retinol < 0.7 $\mu$ M.

- Extremely rare in US, but affects millions of people especially in regions of Africa and South Asia.
- Symptoms include night blindness and very dry, rough skin may indicate a lack of vitamin A. Other signs might include decreased resistance to infections, faulty tooth development and slower bone growth.
- Low vitamin A intake is associated with measles and more severe infectious diseases, including HIV. The infectious process also lowers vitamin A.
- Vitamin A supplements have reduced child mortality by 20-30% in some low- and middle-income countries (BMJ, Aug 25, 2011, 343:d5094. doi: 10.1136/bmj.d5094).

### 7. **Daily requirement** – Daily value (DV) = 5000 IU. UL = 10,000 IU (~ 3000 $\mu$ g) -1 $\mu$ g retinol is about 3.3 IU). Lowest ratio of UL:DV among fat soluble vitamins.

- 1 retinol activity equivalent (RAE) = 1  $\mu$ g *all trans* retinol = 2  $\mu$ g *all trans*  $\beta$ -carotene in oil (a highly absorbable form) = 12  $\mu$ g food-based *all trans*  $\beta$ -carotene = 24  $\mu$ g other mixed dietary carotenoids. Supplements usually provide vitamin A as the acetate ester.
- Adult RDA = 900  $\mu$ g retinol equivalents (~ 2900 IU) for males, 700  $\mu$ g/d (~ 2300 IU) for females and 1300  $\mu$ g/d (~4000 IU) for breast-feeding mothers.

## 8. Uses

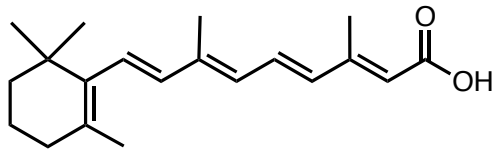
- a. Carotenoids have antioxidant/free radical scavenger activity.
  - Lycopene, a carotenoid (diets rich in e.g. tomatoes, watermelon, red grapefruit), with no vitamin A activity, may have benefit in preventing prostate cancer; doses of 6-30 mg.
  - Luteine, a carotenoid (diets rich in e.g. broccoli, spinach, and kale) with no vitamin A activity, may help prevent macular degeneration; doses of 7-20 mg.
  - Most multivitamins provide only a fraction of these amounts.
  
- b. Cancer - retinoic acid is important for promoting cell differentiation and inhibiting cell proliferation.
  - There is an association of low carotene intake and increased risk of lung cancer in smokers. However, supplementation of  $\beta$ -carotene to smokers (and even previous smokers) resulted in an **increased** risk of lung cancer (Int. J. Cancer 127:172, 2010).
  - Other studies indicate no protective effect of vitamin A against breast or ovarian cancers.
  - Tretinoin (all trans retinoic acid) used in patients with acute promyelocytic leukemia, acts through induction of terminal differentiation.
  
- c. Skin conditions- preserves epithelial morphology.
  - Acne -- topically as retinoic acid. Systemically as 13-cis retinoic acid (isotretinoin) Accutane® Roche.
  - Psoriasis – tretinate (Tegison®)  $\leftrightarrow$  acitretin (Soriatane®)
  - **Warning:** these retinoids are strong teratogens if taken orally.

### IPLEDGE Program

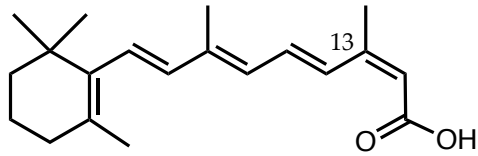
- Between 1982 and 2003 >150 cases of birth defects were documented in the US.
- Types of birth defects included exencephaly, craniofacial abnormalities and eye and cardiac defects.
- IPLEDGE instituted in mid-2000s – an on-line registry system and automated phone system to mitigate the risk associated with taking isotretinoin.
- Patients must demonstrate an understanding of the risks, use multiple methods of contraception and have two clear doctor-administered pregnancy tests before they can obtain the drug.
- Isotretinoin (Rx Accutane) may only be dispensed at authorized US pharmacies that are registered with the IPLEDGE program.

Vitamin A-related Drugs - Three generations – all used to treat severe skin conditions and some cancers, e.g. PML, Kaposi's sarcoma, T-cell lymphoma.

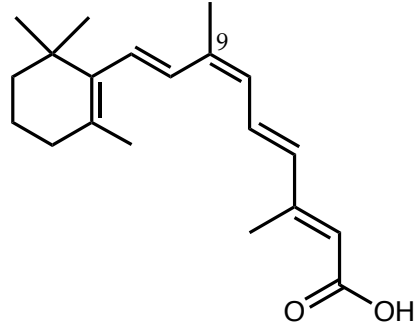
### 1st



all-*trans* Retinoic Acid  
TRETINOIN

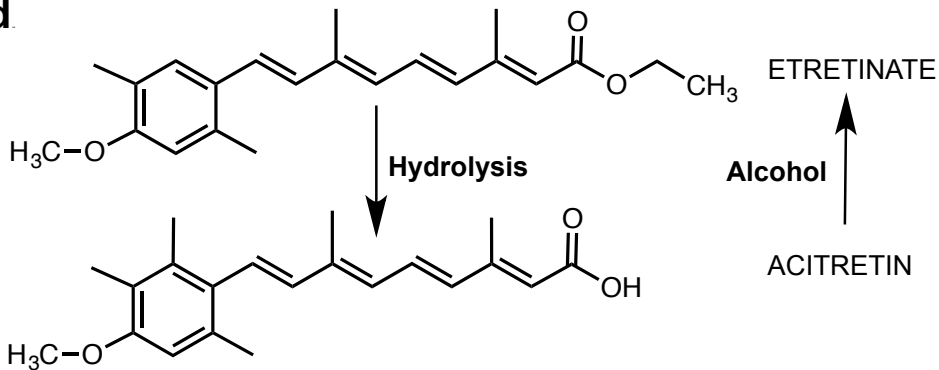


13-*cis* Retinoic Acid  
ISOTRETINOIN

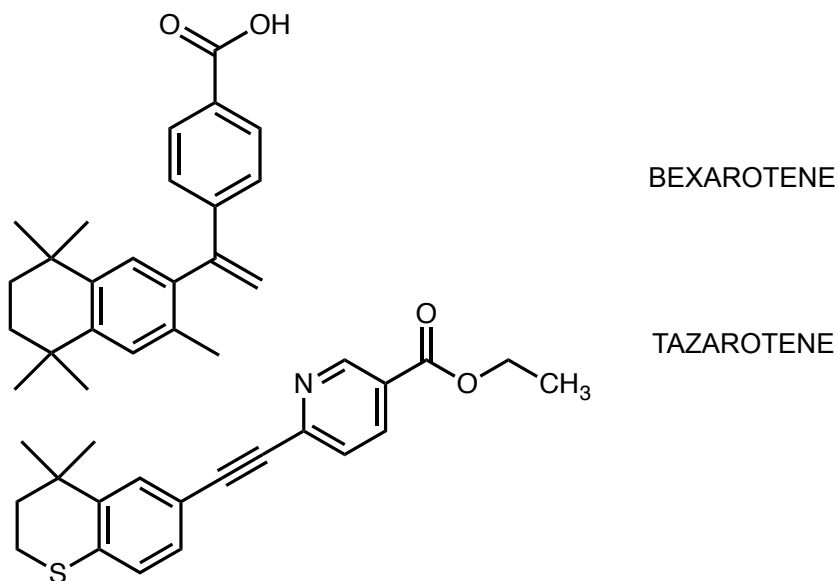


9-*cis* Retinoic Acid  
ALITRETINOIN

### 2nd



### 3rd



BEXAROTENE

TAZAROTENE



## 9. Toxicity

- a. Teratogenic when taken in as retinyl palmitate/acetate in supplements??
  - This is controversial. Significant increase in the risk (1:57) of birth defects has been observed at (performed) vitamin A doses above 10,000 I.U./day taken before the seventh week of gestation (Rothmann et al., NEJM, 1995) – single study.
  - Others suggest no problem at doses of 30,000 IU/day.
  - Additional concern over excessive liver consumption by pregnant women.
  
- b. Hypervitaminosis A is 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.
  - Cod liver oil has about 5,000 IU/5 ml. Beef liver has about 30,000 IU/3 oz.
  - In 1912, the Antarctic explorer, Xavier Mertz, died of vitamin A poisoning from ingesting sled dog liver after supplies were lost in a crevasse.
  - Watch out for polar bear liver -- has 20,000 to 30,000 I.U./g!!!
  - Hypercarotenosis -- eat too many carrots, turn yellow, but no harm done.
  
- c. Risk for bone fractures – an intake of retinol from all diet and supplements over ~10,000 IU has been associated with increased risk for fractures in men and women.

## 10. Consumer Counseling and Advice

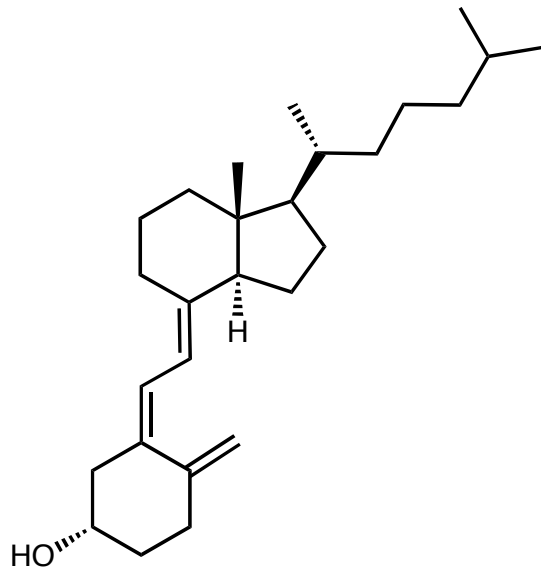
- a. Avoid doses over 5000 IU/d of retinol.
  
- b. Avoid frequent eating of liver and routine use of cod liver oil, especially during pregnancy.
  
- c. Avoid  $\beta$ -carotene as a single dietary supplement, especially for smokers. The amount in multivitamins is usually low.
  
- d. Veggies are the best way to get needed amounts of vitamin A because carotenoid conversion to retinal by  $\beta$ -carotene monooxygenase is regulated by the body's needs (i.e. retinoic acid exerts feedback regulation on the enzyme), so little danger of toxicity if intake is excessively high.

# Vitamin D

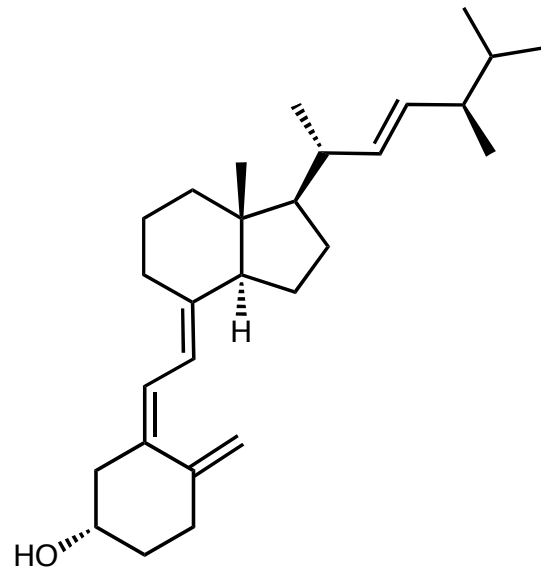
## 1. Background

- Vitamin D has existed on earth for at least 500 million years, produced then in ocean-dwelling phytoplankton exposed to sunlight for photosynthesis. Might have functioned as the first 'sunscreen' or as a photochemical signaling molecule.
- Vitamin D is the anti-rachitic factor present in cod liver oil and after exposure to sunlight, which was known as early as the mid 1800s.
- Rickets is a bone-deforming disease typified by bowed legs and enlargement of the epiphyses of the long bones and rib cage.
  - By the latter part of the 19<sup>th</sup> century, up to 90% of children in industrialized Western Europe had rickets, and it became abundantly clear that rickets was associated with crowded, polluted cities that had grown out of the industrial revolution.
  - Deformed pelvic bones in rachitic women of childbearing age led to the introduction of Caesarean sections as a common medical practice dating from the 1900s.
  - As early as 1827, cod liver oil was recognized to be an effective treatment for controlling rickets. Interestingly, strongly heated/denatured cod liver oil still cured rickets, so it was not the vitamin A component.
- In the 1920s, it was found that rickets could be treated with UV radiation. Importantly, it was found that irradiating one arm of a rachitic child cured rickets everywhere in the body, so this could not be just a 'local' phototherapy effect – a hormone?
- A hormone is a chemical substance produced by the cells of one tissue and conveyed by the bloodstream to another tissue where it exerts its physiological function.
- Technically, vitamin D is not a vitamin. It is the name given to a group of fat-soluble *pro-hormones* (substances that are precursors to hormones, and which usually have little hormonal activity by themselves), i.e. vitamins D2 and D3 are pro-hormones that are converted to the active hormone, 1,25-dihydroxy vitamin D3.
- Principal physiological function of (activated) vitamin D in human is to maintain serum calcium and phosphate concentrations in a range that maintains critical cellular processes, like neuromuscular function and bone ossification.

Two main forms; Vitamins D2 and D3



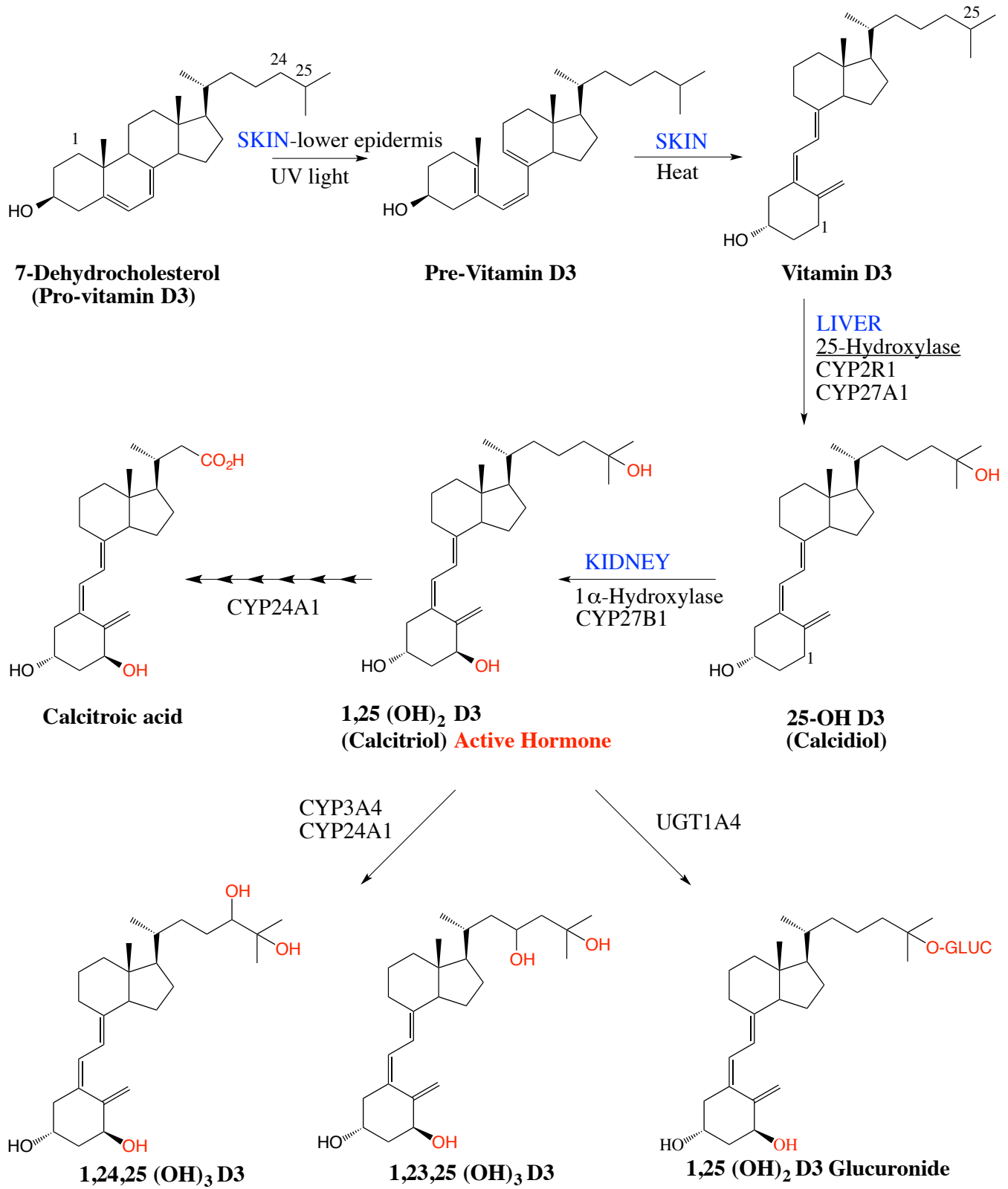
**D3 - Cholecalciferol**



**D2 - Ergocalciferol**

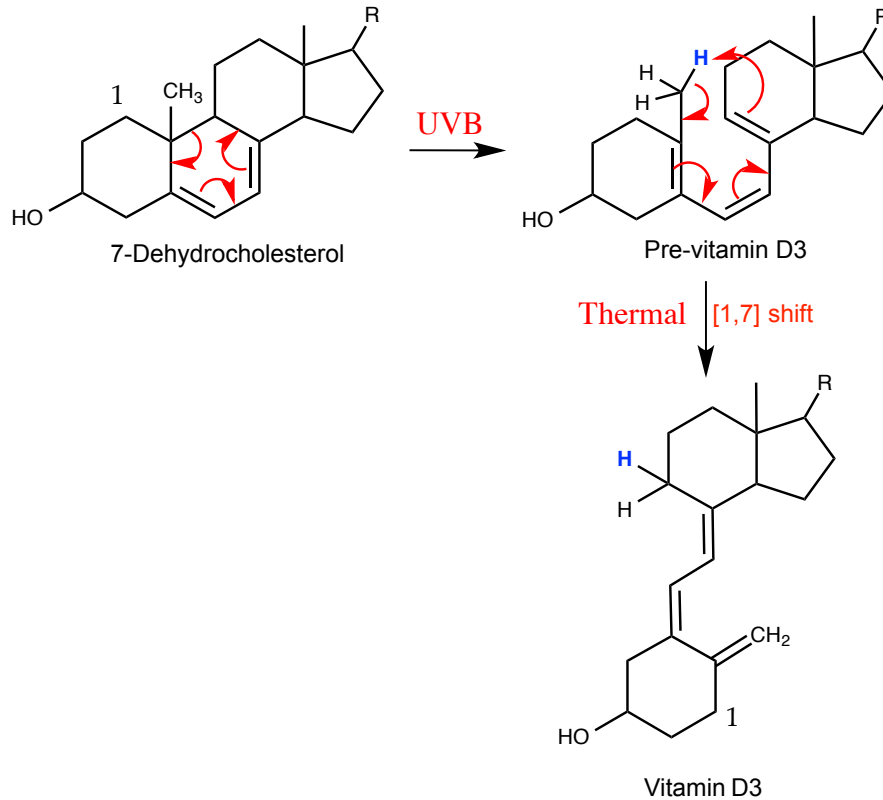
- Two major forms of vitamin D important to humans are D2 – ergocalciferol, found naturally in plants), and D3 – cholecalciferol, made naturally in the body when the skin is exposed to UVB radiation in sunlight.
- Vitamin D1 was a mixture of vitamin D2 and lumisterol (a photochemical degradation product of ergosterol).
- Vitamins D2 and D3 are secosteroids, derived from ergosterol and 7-dehydrocholesterol, respectively. These vitamins are very similar in structure to the normal tetracyclic steroid nucleus, but one of the rings is incomplete.
- Controversy has long existed about the relative potency of D2 versus D3. In humans, a case can be made for using only D3 because:
  - ✓ D3 is more effective at increasing circulating concentrations of 25-OH D,
  - ✓ D3 metabolites bind more strongly to the vitamin D receptor,
  - ✓ D2 has a shorter shelf life.

## 2. Synthesis, Metabolic Activation and Inactivation of Vitamin D3



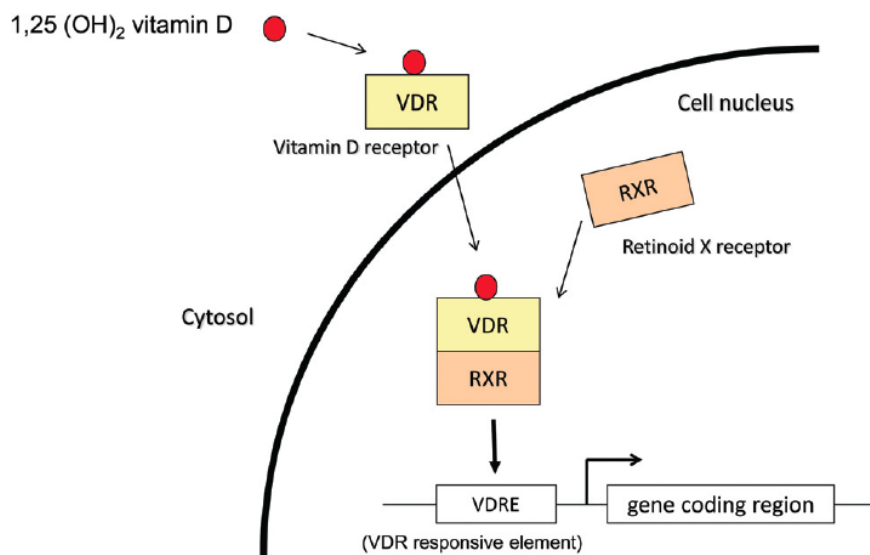
## Photoactivation of 7-Dehydrocholesterol

- Occurs maximally around 295 nm which requires sun angle  $>45^\circ$  above horizon. This almost never happens at high latitudes.

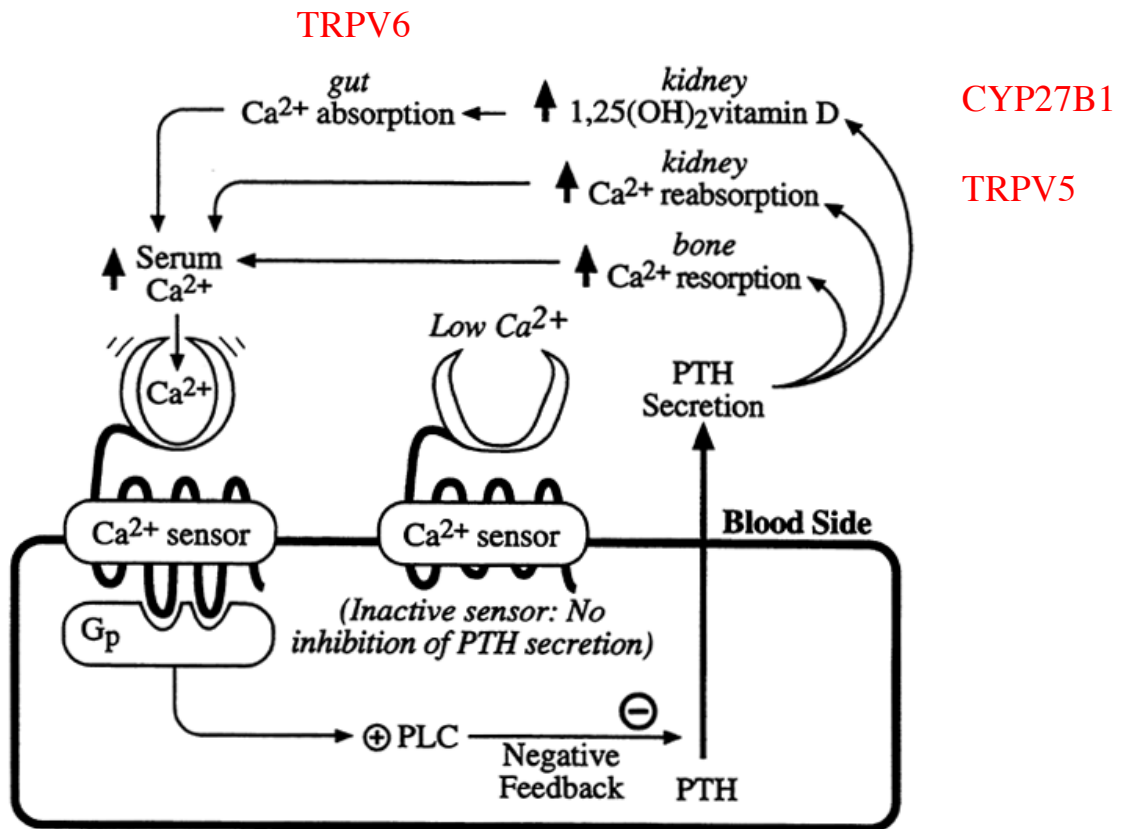


### 3. Function

- 1,25 DHCC, the hormonally active form of vitamin D, mediates its biological effects by binding to the vitamin D receptor (VDR) - a steroid hormone receptor.



- Upon translocation to the nucleus VDR heterodimerizes with RXR and binds to specific response elements in the promoter region of vitamin D responsive genes, such as calbindin (Ca binding protein) and osteocalcin/osteopontin (bone-forming proteins).
- VDR activation enhances gene expression of  $\text{Ca}^{2+}$  binding and transport proteins involved in  $\text{Ca}^{2+}$  (and phosphate) absorption in the intestine and in  $\text{Ca}^{2+}$  reabsorption from the kidney.
- Calcium is essential for **healthy teeth/bones**, blood clotting, synaptic transmission, and muscle function.
- Vitamin D acts in concert with parathyroid hormone (PTH) to control calcium homeostasis.



- VDR activation in bone modulates bone mineralization.
- In kidney failure, renal synthesis of activated vitamin D and renal reabsorption of calcium both decrease, resulting in low serum calcium levels and increased PTH secretion. Excessive bone resorption can cause **metabolic bone disease** in renal failure.

- VDR signaling is also involved in **modulating cell proliferation and differentiation**.
  - ✓ Numerous clinical studies have been published that suggest that a high intake of vitamin D may reduce the risk of certain types of cancer, notably colorectal cancer, and possibly breast, prostate and pancreatic cancers.
  - ✓ Laboratory studies have shown that calcitriol promotes cellular differentiation, decreases cancer cell growth and stimulates apoptosis - 'programmed cell death'.
  - ✓ Overall, however, the data are inconsistent regarding a protective effect of vitamin D against any specific type of cancer. Interestingly, there is an overall small association of vitamin D supplement use and decreased death due to all causes.

#### 4. Sources

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

#### 5. Requirements - DV = 400 IU; UL = 4000 IU.

- In late 2010, IoM increased their RDA to 600 IU/day for people age 1-70 yrs (800 IU if >70 yrs) and the UL to 4000 IU (from 2000 IU). [1 microgram = 40 IU].

#### 6. Toxicity

- As with Vitamin A, vitamin D overdose typically happens over a period of time rather than from a single large dose.
- For children under the age of 12 months, a sustained intake of 1,000 mg (40,000 IU) a day will produce severe toxicity (i.e. calcification of soft tissues such as the lung, kidney) in one to four months.
- For adults, 2,500 mg (100,000 IU) a day can result in toxicity in a few months.

#### 7. Deficiency state

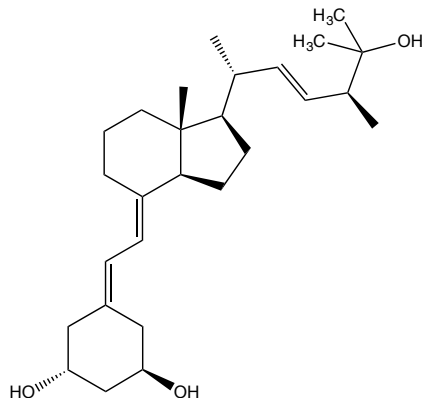
- Assessed on basis of plasma levels of 25-OH D3. Natural levels in adults who live or work in the sun are 50-70 ng/ml.
- Minimum level needed to prevent rickets and osteomalacia is 15 ng/ml.
- Deficiency commonly assessed as levels <20 ng/ml.

## 8. At risk for deficiency

- Infants/Elderly with minimal sun
- Dark skin with minimal sun
- Religions that require the entire body be covered
- Fat malabsorption
- Inflammatory bowel diseases
- Kidney failure
- Seizure disorders treated with anticonvulsants, which increase 1,25 DHCC elimination by CYP3A4 pathways.

## 9. Uses – the importance of adequate intake and (perhaps) the value of using supplements of this vitamin is now beginning to be realized.

- Deficiencies due to low sun exposure → osteomalacia and osteoporosis.
- 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.
- Renal failure – uremic patients cannot synthesize 1,25 DHCC. Resultant hypocalcemia and secondary hyperparathyroidism are a major cause of metabolic bone disease occurring in kidney failure.
  - ✓ Rocaltrol and generic products are used to provide this active metabolite directly. Available in capsules and as an oral solution, 0.25 – 0.5 mg.
  - ✓ Also Paricalcitol (Zemplar)- Modification of 1,25 DHCC used orally for hyperparathyroidism. 1 mg three times per week.



## 10. Consumer Counseling and Advice

- Assure intake of at least 400 IU/d. Multivitamins usually contain this amount.
- There is evidence that more than 400 IU/d may be beneficial if sun exposure is minimal; 800 IU/d seems optimal based on evidence today.
- Vitamin D is very important for bone health, but also may help reduce risks for cancer and other diseases.
- Postmenopausal women should take a vitamin D supplement as well as calcium supplement.



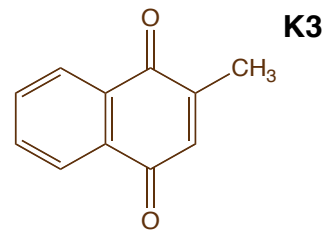
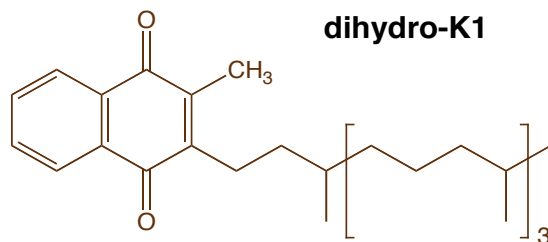
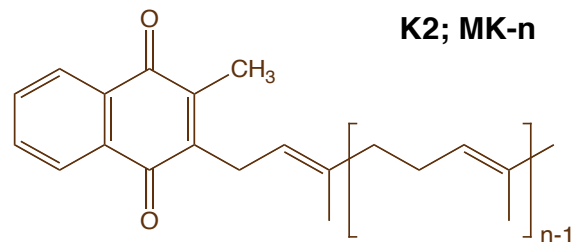
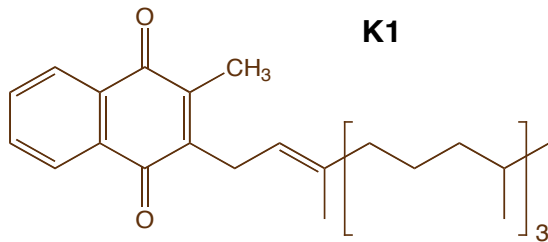
# Vitamin K

## 1. Background

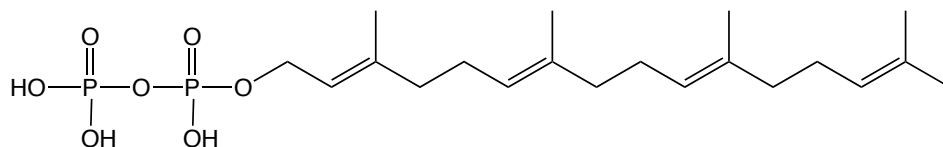
- Vitamin K (the K is for 'Koagulation') was discovered as researchers tried to understand why chickens and other experimental animals fed diets with very low lipid contents developed hemorrhages and why blood taken from these animals clotted slowly.
- In the 1930s, it was found that this hemorrhagic disease could be cured by supplementation with lipid extracts of green plants and fish-meal that had been subjected to bacterial action.
- Soon after, the plant material - vitamin K1 - was isolated from alfalfa, and vitamin K2 was identified as the factor from putrefied fish-meal.

## 2. Structures

- A group of 3-substituted, 2-methyl-1,4-naphthoquinones having anti-hemorrhagic activity.

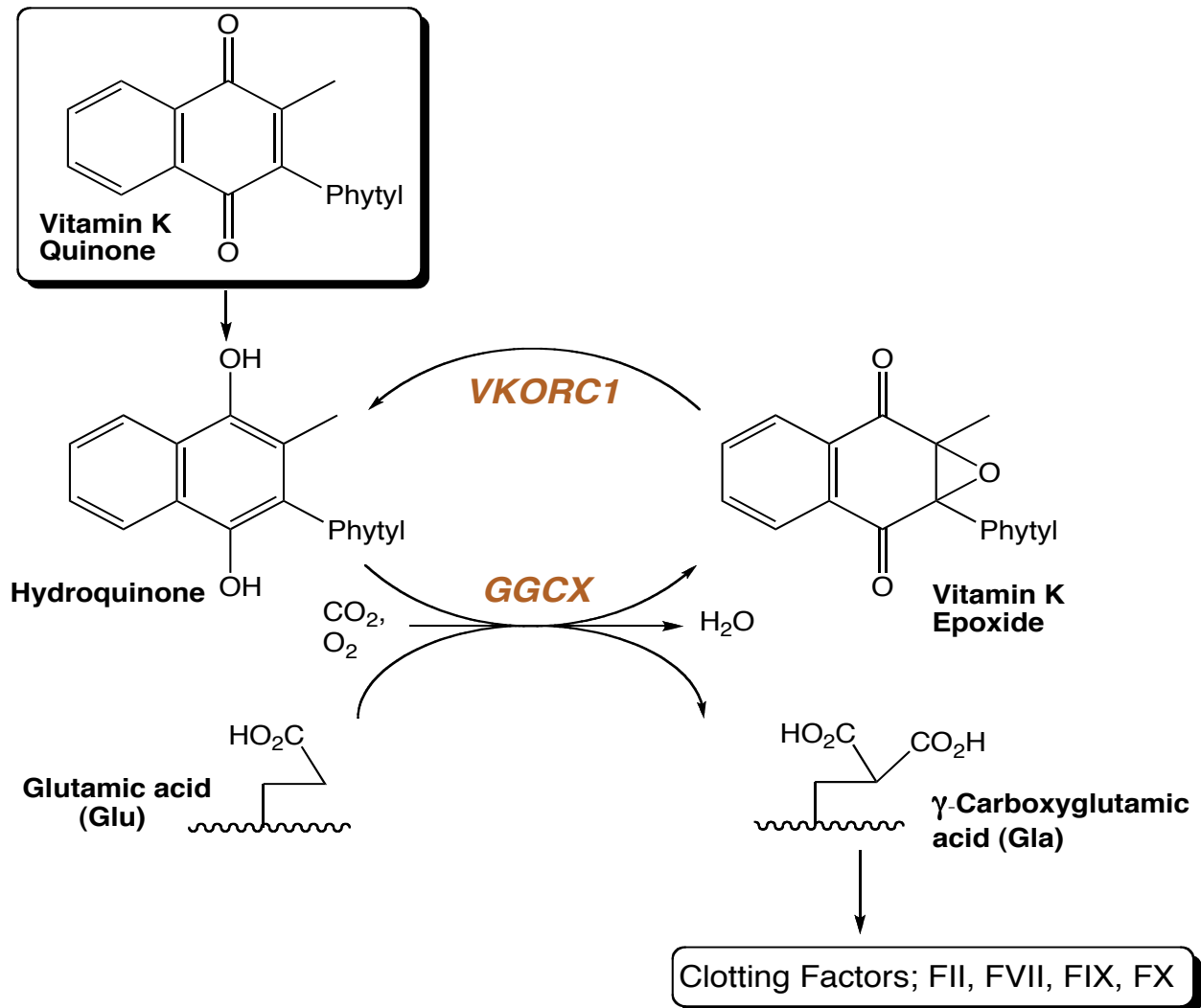


- K1 – Phylloquinone, most prevalent form of vitamin K found at high concentrations in green leafy vegetables.
- 2'-3'-Dihydro-K1 – a form of vitamin K produced during the hydrogenation of vitamin K1-rich vegetable oils.
- K2 – series of Menaquinones, at least 13 known (MK1-MK13).
  - ✓ MK-4, present in certain foods and formed in the body from K3 by reaction with geranylgeranylphosphate.
  - ✓ MK-7, high concentrations in some fermented foods, e.g natto.
  - ✓ MK-8-13, synthesized by bacteria in the gut.
- K3 – Menadione, 'provitamin', lacks the side-chain that is required for vitamin K activity. Can be converted in the body to MK-4 upon reaction with geranylgeranyl diphosphate.

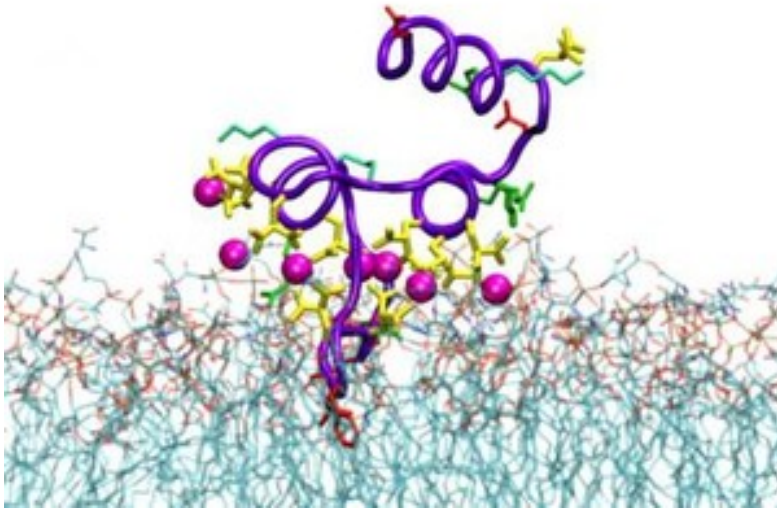


3. **Function** – Vitamin K is the required cofactor for the vitamin K cycle that functions in the post-translational  $\gamma$ -carboxylation of glutamic acid residues (an activation process that forms Gla proteins) on several precursor proteins with important biological functions.

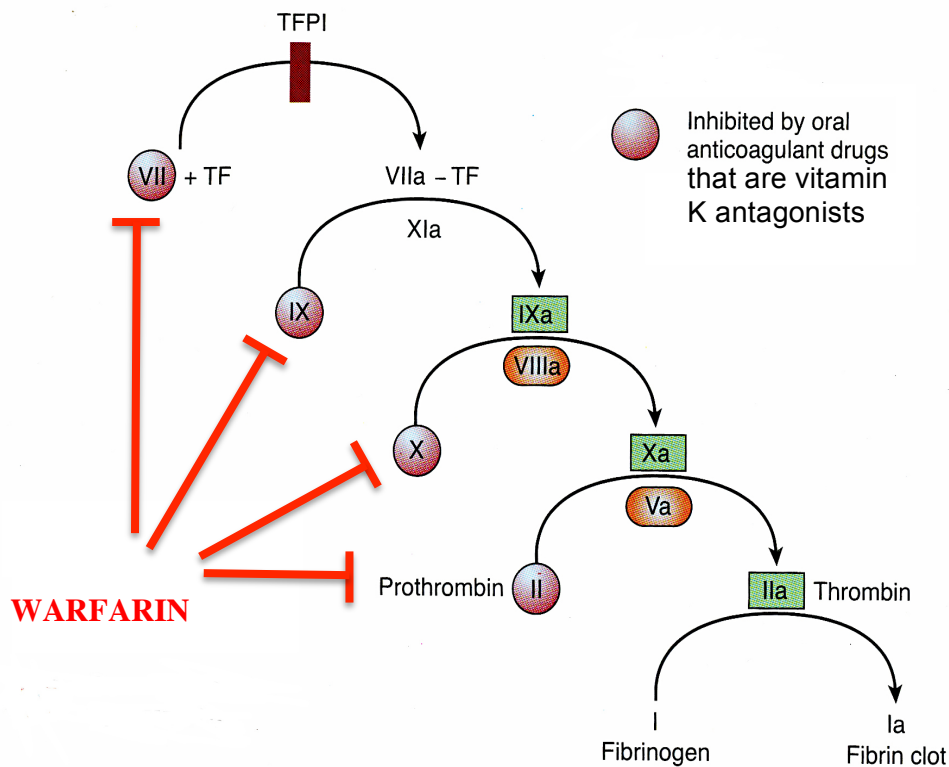
Vitamin K Cycle, Gla Protein formation and Blood Clotting



- *VKORC1* is the gene that encodes the enzyme, vitamin K epoxide reductase enzyme (VKOR), which is the target for warfarin and other vitamin K antagonists.
- Warfarin inhibits VKOR, thereby reducing the recycling of reduced vitamin K in the liver.
- GGCX is the gene encoding the  $\gamma$ -glutamyl carboxylase enzyme that forms the Gla-containing clotting factors.
- The N-terminus of prothrombin contains 10 Glu residues that are all converted to Gla in fully active clotting factor II.



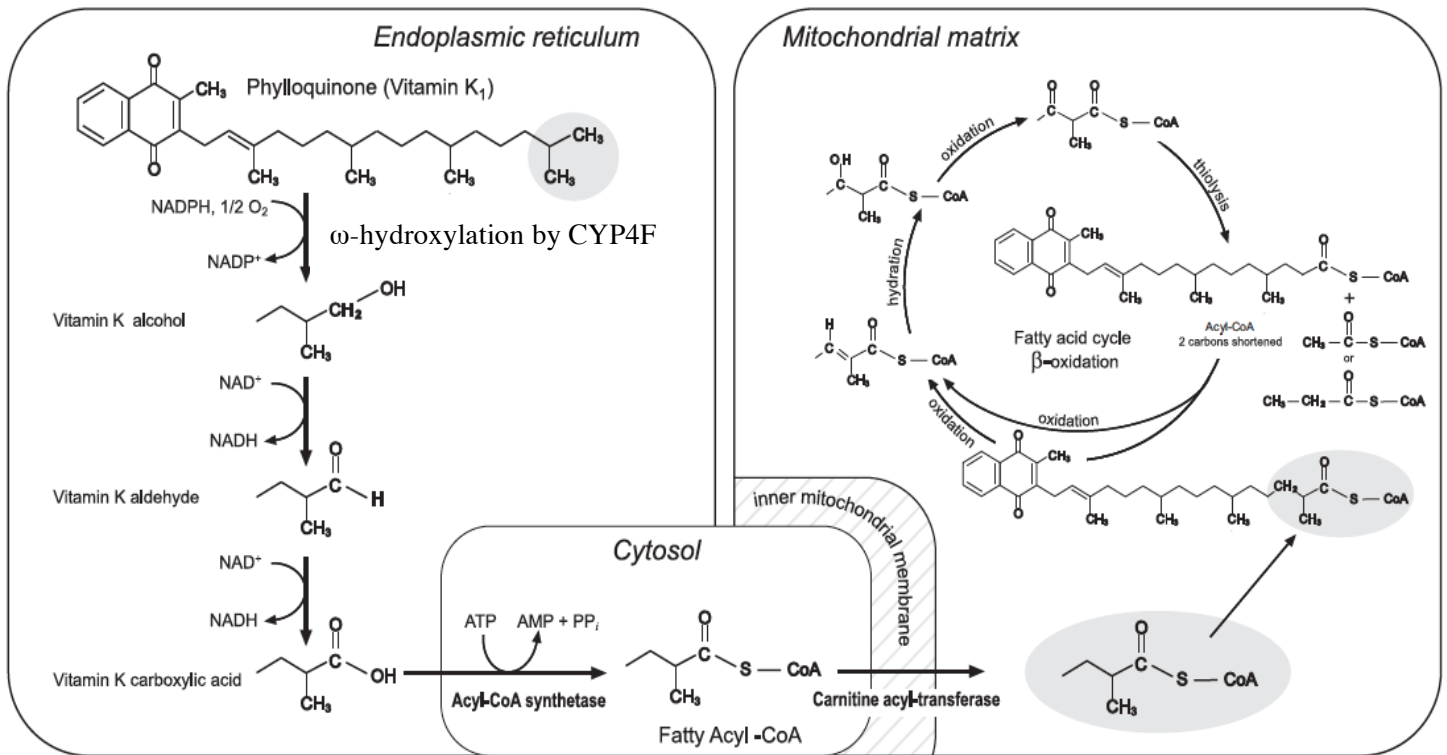
- $\text{Ca}^{2+}$  ions (red) bind to Gla residue clusters (yellow) at the N-terminus of the vitamin K-dependent protein, inducing a conformational change that facilitates Gla protein interactions with phospholipids on the cell surface membrane.



- Note that new direct orally acting anticoagulant drugs (DOACs), such as rivaroxaban (Factor Xa inhibitor) and dabigatran (Factor IIa inhibitor), do NOT act via the vitamin K cycle.

#### 4. Metabolism

- Initiated by P450-mediated  $\omega$ -hydroxylation (CYP4F2 and CYP4F11) with subsequent  $\beta$ -oxidation. [ $-\text{CH}_3 \rightarrow -\text{CH}_2\text{OH} \rightarrow -\text{CO}_2\text{H} \rightarrow -\text{COSCoA}$ ]



- Sulfate and glucuronide conjugates of chain-shortened K acids are excreted in the urine and bile.

#### 5. Deficiency

- Vitamin K deficiency increases spontaneous hemorrhaging. Requires a chronic failure to ingest sufficient plant-derived vitamin K1 or long term antibiotic therapy that presumably eliminates the intestinal flora that produce vitamin K2.
- Both of these sources are routinely described in the literature as contributing equally to vitamin K status. However, it has been argued this overestimates the contribution of bacterial K2 because of poor bioavailability from the lower intestine where the bacteria involved in menaquinone synthesis reside.
- Vitamin K status can be assessed using the PIVKA-II test that measures descarboxy prothrombin with an ELISA test.

## 6. Uses

- Coagulation - For an anticoagulant overdose, use K1 oral, 2.5-5 mg (if INR >9, but no bleeding), if serious bleeding or INR >20, K1 slow i.v., 10 mg (+ fresh plasma).
- K1 is used routinely at birth (i.m. 0.5-1 mg) to prevent neonatal hemorrhage, because:
  - ✓ The placenta transmits lipids and vitamin K relatively poorly.
  - ✓ Breast milk is low in vit. K, (contains about 2.5 µg/L; cow's milk contains 5000 µg/L).
  - ✓ The neonatal gut is sterile during the first few days of life.
- Bone health -Vitamin K participates in  $\gamma$ -carboxylation of osteocalcin required in bone deposition. Use of 25 mg/d for 2 years decreased hip fractures in an older population, but studies are inconclusive about benefit.
- Prevention of vascular calcification – possible emerging role related to  $\gamma$ -carboxylation of MGP (matrix Gla protein), the body's natural calcification inhibitor. Role for dialysis patients?

## 7. Source

- Green leafy vegetables; esp. spinach, collard greens, kale.
- Vitamin K app identifies these as providing >1000 µg vitamin K/cup.

## 8. Dose

- DV is 80 µg. There is no UL.
- DV may be too low for optimal activities as Adequate Intake levels set by IoM are 90-120 µg/day.

## 9. Toxicity - Some allergic reactions reported IV, otherwise nothing special.

## 10. Consumer Counseling

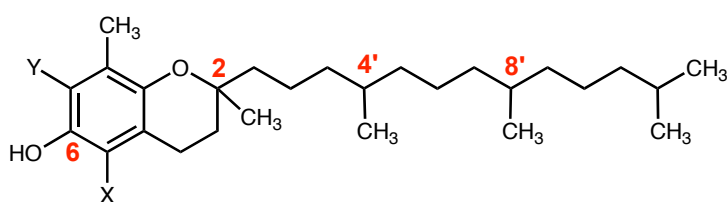
- Adequate intake is important for the ability of blood to clot and for healthy bones.
- A good diet with leafy vegetables (and a healthy gut flora) can probably supply needs but the amount in most multivitamins will assure a good intake.
- If patient on warfarin, then it is important for them to work with health care providers to keep vitamin K intake steady in order to avoid fluctuations in warfarin maintenance dose.

## Vitamin E ('a vitamin looking for a disease')

### 1. Structures

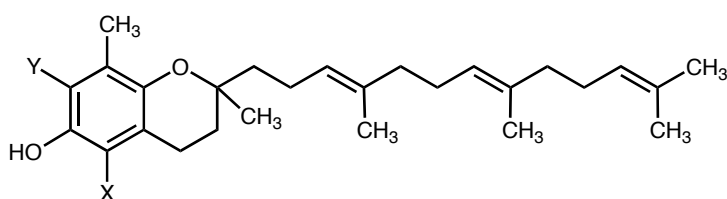
- The term Vitamin E is used for a family of 8 different molecules; four tocopherols and four tocotrienols, all of which have antioxidant properties.
- All feature a **chromanol ring** containing a phenolic hydroxyl group at the 6-position that can donate a hydrogen atom (H<sup>•</sup>) to reduce free radicals and a hydrophobic side-chain which aids penetration of biological membranes.
- The tocopherols have 8 possible stereoisomers. Naturally occurring tocopherols have the R configuration at all three chiral centers, ie 2*R*,4'*R*,8'*R*.

#### Tocopherols



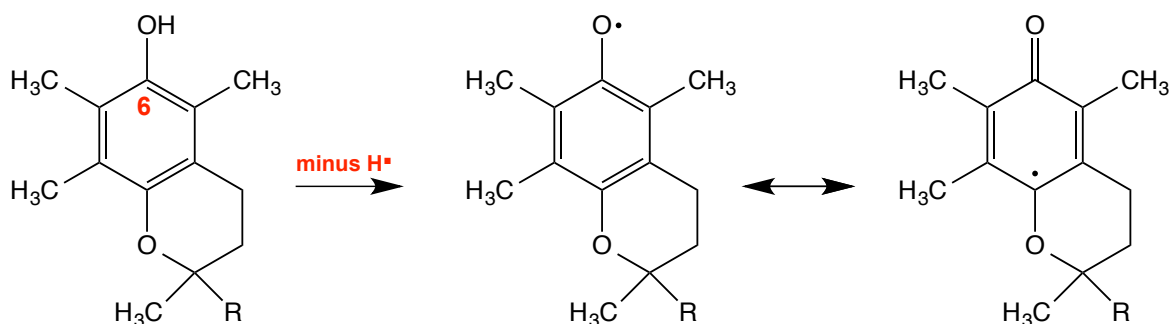
Naturally occurring homologs	X	Y
$\alpha$	CH <sub>3</sub>	CH <sub>3</sub>
$\beta$	CH <sub>3</sub>	H
$\gamma$	H	CH <sub>3</sub>
$\delta$	H	H

#### Tocotrienols



### 2. Antioxidant properties

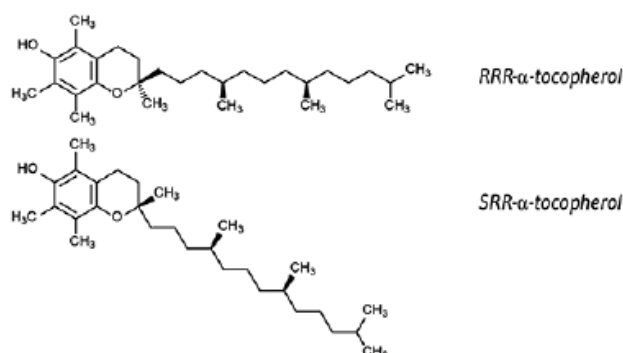
- Vitamin E has an important function as an antioxidant. One electron oxidation of  $\alpha$ -tocopherol leads to the resonance stabilized radical shown below. Facile donation of H<sup>•</sup> to peroxy radicals (ROO<sup>•</sup>) neutralizes them and terminates lipid peroxidation at the propagation step (see p.25 for details).



- As a consequence, vitamin E is an excellent chain breaking, free radical scavenger that prevents the propagation of free radical damage in biological membranes thus preserving essential membrane function.
- The antioxidant potency depends on the substitution pattern of the methyl groups on the aromatic ring.  $\alpha$ -Tocopherol is the most potent and  $\delta$ -tocopherol the least potent antioxidant in vitro.

### 3. Pharmacological activity and transport

- $\alpha$ -Tocopherol is the most important form of vitamin E. As assessed by the rat resorption-gestation test, *RRR*- $\alpha$ -tocopherol is the most biologically potent stereoisomer.
- Only stereoisomers with the 2*R*-configuration are considered to contribute to satisfying vitamin E requirements in humans.
- Neither  $\beta$ -,  $\gamma$ -,  $\delta$ -tocopherol nor the tocotrienols contribute to the body's vitamin E requirement because, although absorbed, are poorly recognized by the  $\alpha$ -tocopherol transport protein ( $\alpha$ TTP) in the liver.



- $\alpha$ TTP is responsible for the selective transfer of (2*R*)- $\alpha$ -tocopherol into VLDL (with subsequent distribution to other serum lipoproteins). Other vitamin E forms are also better metabolized (CYP4F2), so are not conserved in the body.

### 4. Daily requirement

- DV = 30 I.U (20 mg natural).
  - *RRR*- $\alpha$ -tocopherol (natural) 1 mg = 1.5 I.U.
  - *all-rac*- $\alpha$ -tocopherol (synthetic) 1 mg = 1.1 I.U.
  - *all-rac*- $\alpha$ -tocopherol acetate (synthetic) 1 mg = 1.0 I.U.
- UL = 1000 mg (1500 IU natural)

### 5. Dietary sources

- Almost ubiquitous; rich sources are wheat germ and sunflower seed oils, green vegetables, whole grain cereals.
- Fortified margarine supplies represent much of our intake in the U.S.

### 6. Deficiency state

- Rare (in developed countries), usually due to fat malabsorption.
- 'Tokos' is Greek for birth. Deficiency in rats causes sterility in male rats and fetal resorption in pregnant females.

- In adult humans, deficiency is generally characterized by neuromuscular abnormalities and myopathies. These peripheral neuropathies are considered to be due to free radical damage to nerves.
- In premature infants, a deficiency state (often characterized by hemolytic anemia, fragile RBCs damaged by free radicals) has been described wherein stores of vitamin E are low at birth due to poor placental transport.
- Diagnosis is based either on measuring the ratio of plasma  $\alpha$ -tocopherol to total plasma lipids ( $< 0.8$  mg/g), or having a plasma level  $< 20$   $\mu$ M.

## 7. Toxicity

- Tocopherols are generally considered non-toxic.
- Bleeding can be an adverse effect, but this is rare at doses less than 1000 mg/day.
- Exacerbated bleeding when given together with warfarin is the most significant drug interaction involving Vitamin E.
- Bleeding mechanism has been suggested to involve:
  - ✓ Inhibition of the  $\gamma$ -carboxylase enzyme (GGCX) in the vitamin K cycle by vitamin E metabolite(s).
  - ✓ Direct effect on platelet function may also contribute.

## 8. Uses -- The claims for benefit of supplements of vitamin E are numerous and include decreasing heart disease, cancer, dementia and prolongation of life – many of which seem plausible benefits of the antioxidant properties of vitamin E.

- Cancer – 600 IU every other day provided no overall benefit in cancer risk among healthy women (JAMA, 2005:294:56).
- Alzheimers Disease -- high doses (2000 IU/d) showed some benefit in slowing progression, but not in prevention.
- Retrolental fibroplasia and bronchopulmonary dysplasia. Eye and lung damage in premature infants on oxygen. I.V. vitamin E (MVI Pediatric, Astra] seems to offer some protection. [Fat soluble vitamins ‘emulsified’ with polysorbate 80 to render them suitable for injection]

## 9. Consumer Counseling and Advice

- The amount in a multivitamin is probably adequate (30 IU) for most.
- Natural vitamin E (RRR) is better utilized than the synthetic racemate.
- Health benefits from high dose vitamin E supplements do not seem to have materialized.
- Use vitamin E supplementation cautiously if there is any tendency to bleed easily.



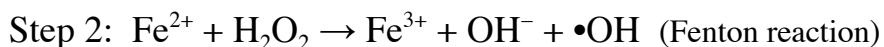
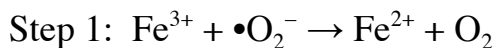
## OXIDATIVE STRESS AND PROTECTIVE MECHANISMS THAT INVOLVE VITAMINS (and MINERALS)

Focus here is on:

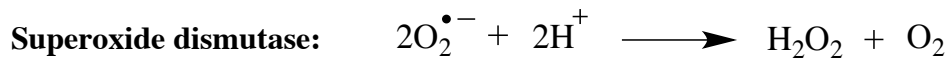
- Minerals like Fe, Cu and Zn, which are important for enzymes that detoxify reactive oxygen species (ROS).
- Vitamins and minerals (Se) in the glutathione pathway that detoxifies (lipid) peroxides
- The synergy between vitamins E and C in scavenging of (lipid) radicals.

### Oxidative stress

- Oxygen is essential to life, but obscures the fact that it is also a poison and aerobes survive only because they have evolved antioxidant defenses.
- The oxidative status of cells is determined by the balance between *antioxidants* and *pro-oxidants*.
- The major classes of *pro-oxidants* are ROS and reactive nitrogen species (RNS). ROS/RNS is a collective term that includes both radicals and certain non-radicals that are oxidizing agents and/or easily converted into radicals.
- Examples of ROS include:
  - ✓ Superoxide anion [O<sub>2</sub><sup>-•</sup>]
  - ✓ Hydroxyl radical [OH<sup>•</sup>]
  - ✓ Hydrogen peroxide [H<sub>2</sub>O<sub>2</sub>]
  - ✓ Peroxyl radicals [ROO<sup>•</sup>]
- The Haber-Weiss reaction generates hydroxyl radical from superoxide and hydrogen peroxide in two steps catalyzed by iron.



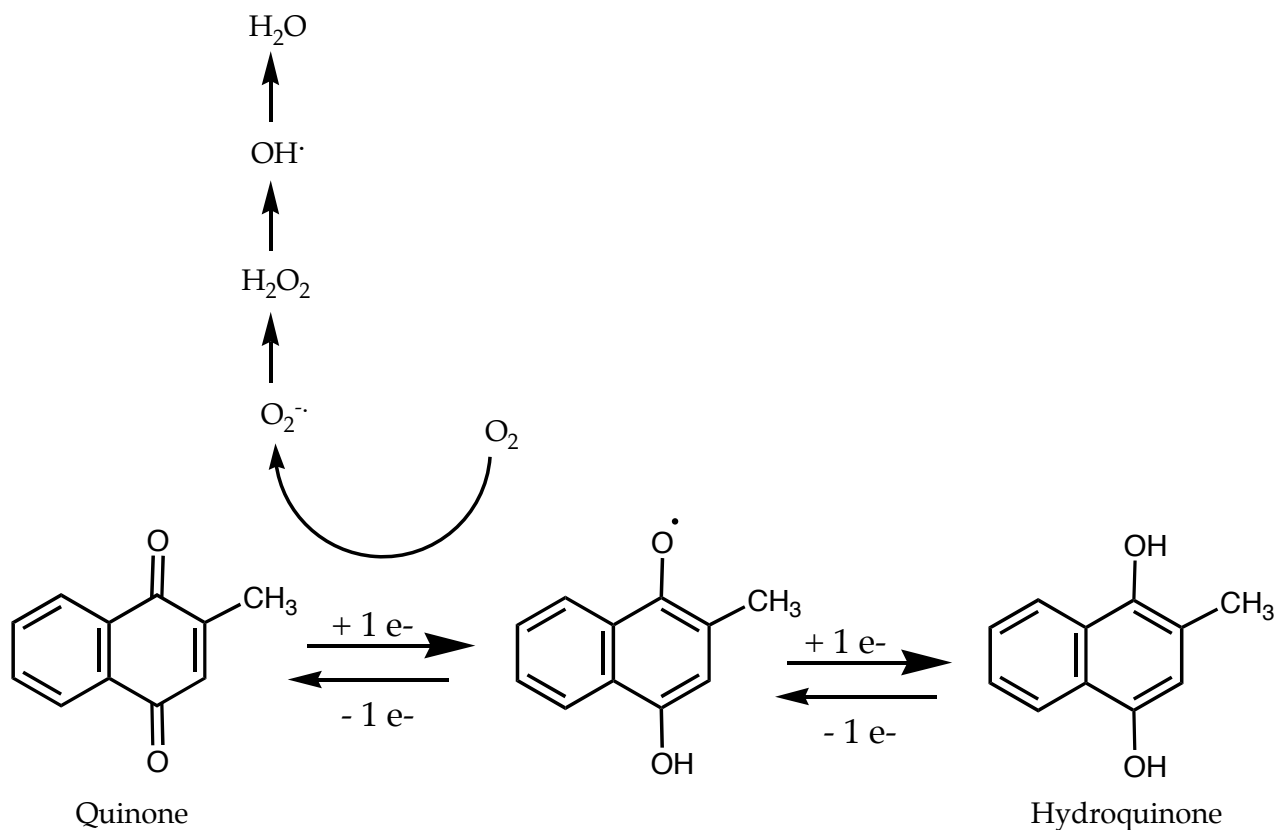
Enzymes that degrade hydrogen peroxide and superoxide.



- SOD in mitochondria has a **Mn** cofactor, whereas cytosolic SOD uses **Cu and Zn**.
- Catalase is a heme-containing protein and so needs **Fe**.

Futile Cycling

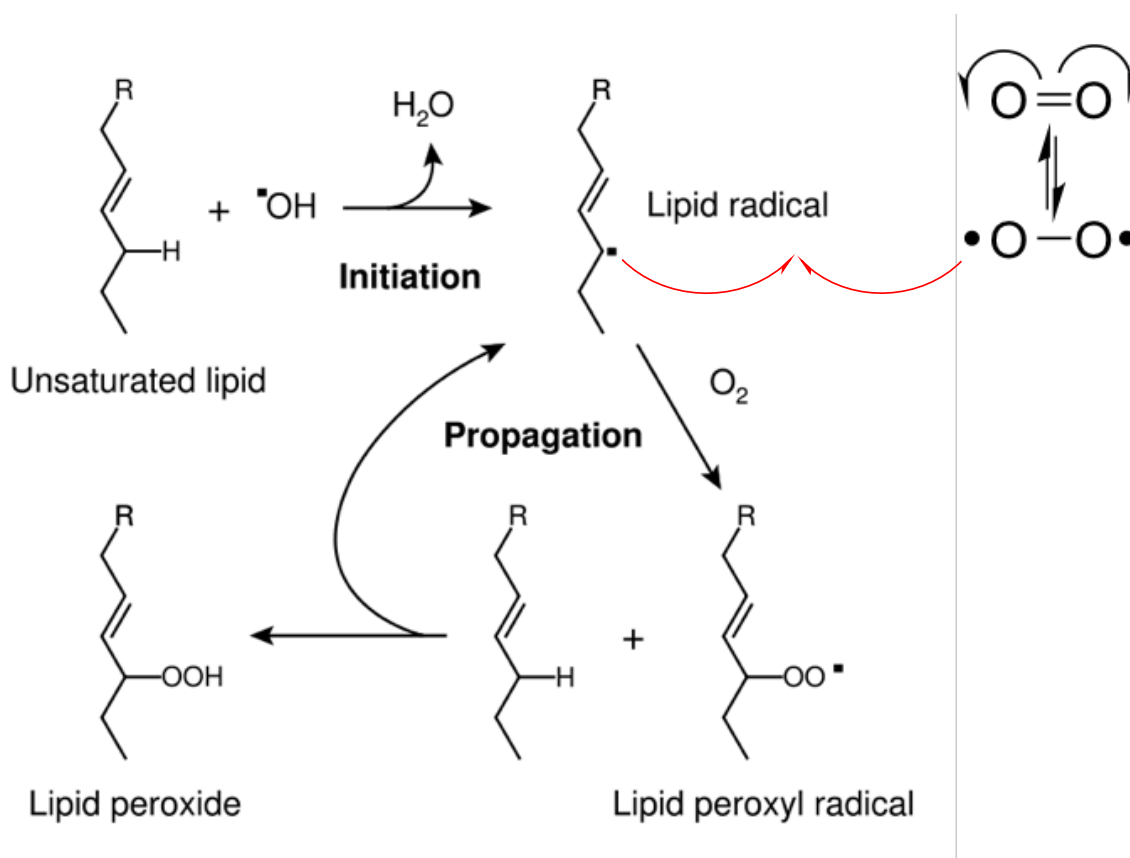
- ROS are also generated during 'futile cycling' of, for example, quinones
- Sequential additions of single electrons to molecular oxygen generate superoxide anion, hydrogen peroxide and hydroxyl radical.



- Other sources of e<sup>-</sup>: Reduction of nitroaromatics, mitochondrial respiration, UV light radiation,

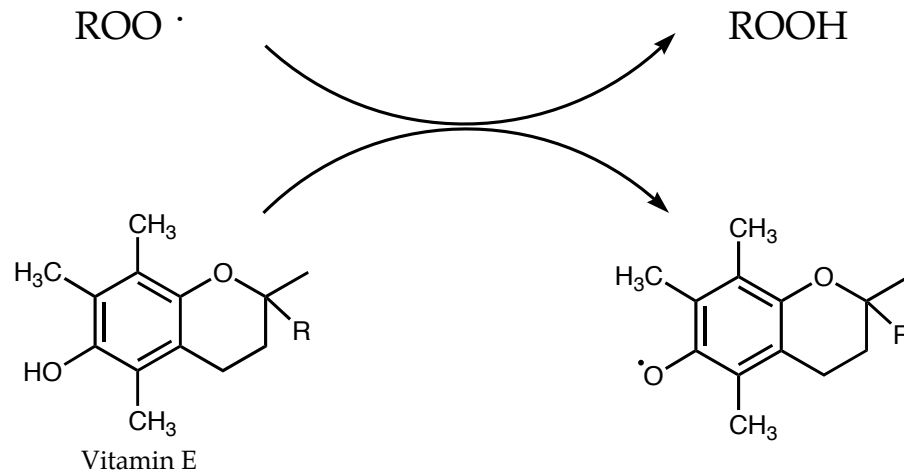
**Targets of ROS** - DNA, thiols, enzymes, membranes, collagen, lipids, e.g., unsaturated lipid.

- ROS causes tissue damage through promotion of lipid peroxidation with subsequent damage to biological membranes. Important in; inflammation, carcinogenesis, hemolysis, atherosclerosis, arthritis, aging, adverse drug effects (ROS from futile cycling of quinones and nitroaromatic-containing drugs).

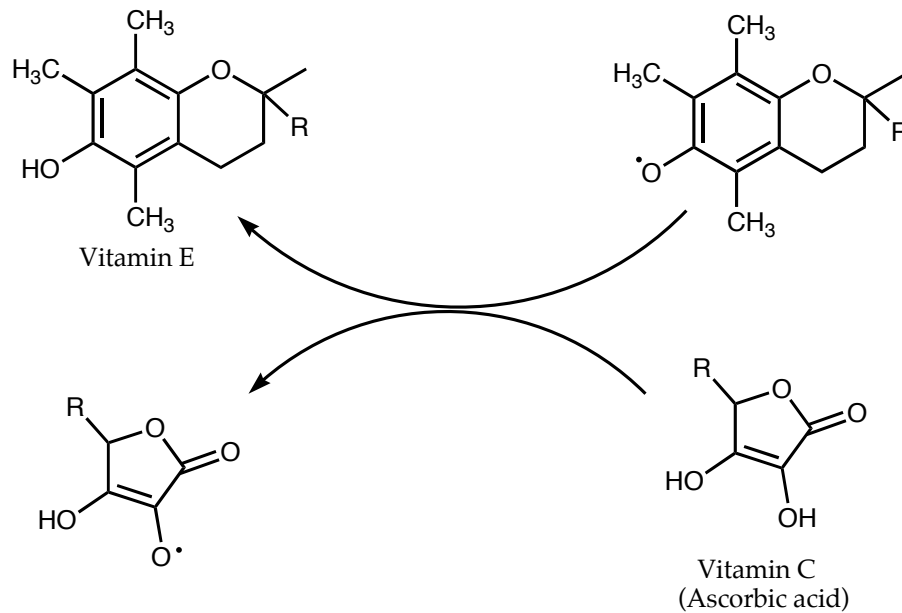


- Lipid peroxidation is a well-known example of oxidative damage to cell membranes and other lipoprotein structures that can be quenched through the protective actions of various antioxidant processes that have evolved to combat oxidative stress.
- The lipid peroxidation chain reaction can be terminated at the propagation step by reaction of the lipid peroxy radical ( $\text{ROO}\cdot$ ) with vitamin E, instead of oxygen.
- Remember, Vitamin E is the main lipophilic, chain-breaking antioxidant present in cell membranes – see page 22.

### Vitamin E 'soaking up' a lipid peroxy radical



### Regeneration of Vitamin E by Ascorbate

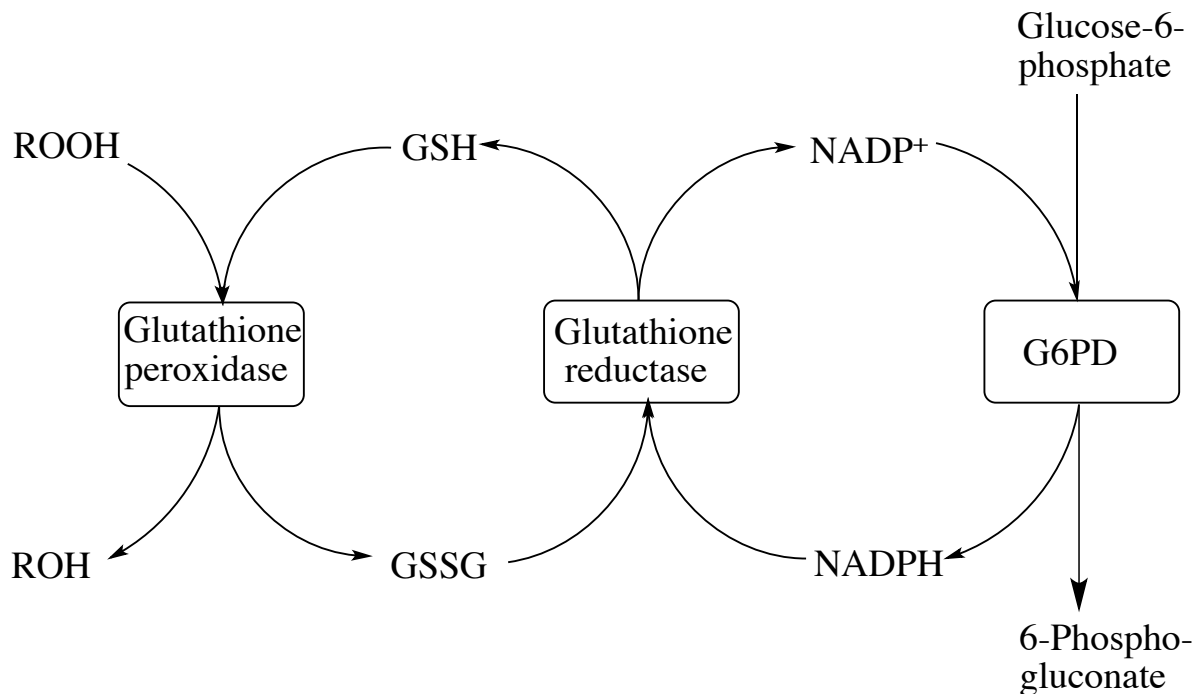


- The antioxidant action of vitamin E is enhanced by vitamin C, which can react with the resulting oxygen-centered vitamin E radical to regenerate vitamin E.
- Antioxidant clinical trials involving vitamin E have been very disappointing and may have failed for several reasons;
  - ✓ too low a dose, too short a duration
  - ✓ inadequate monitoring of vitamin intake from diet; carotenoids, vitamin E, vitamin C
  - ✓ lack of inclusion of vitamin C

## Other Protective Mechanisms

### *Glutathione pathway*

- Neutral lipid hydroperoxides are not completely benign. For example, being more polar than the parent lipids, they can perturb membrane structure/function and be damaging on that basis alone. The glutathione pathway provides a means for protection *via* reductions that are reliant on NADPH produced by G6PD in the phosphogluconate pathway, and on reduced glutathione (GSH).



- G-6-P Dehydrogenase (G6PD) is a key enzyme controlling reducing power in cells.
- G6PD is particularly important in red blood cells, where oxygen tension is high.
- G6PDH deficiency is the most common genetic defect in the world affecting 400 million people of African and Mediterranean descent primarily. Defective enzyme causes oxidative stress, often seen as hemolytic anemia.
- The glutathione pathway depends on an adequate supply of;
  - ✓ the mineral, selenium, for glutathione peroxidase
  - ✓ vitamin B2 (riboflavin), the cofactor for glutathione reductase
  - ✓ vitamin B3 (niacin), to maintain cellular concentrations of NADP(H).

Summary of pathways interacting to combat oxidative stress due to peroxy radicals

