

Vitamin K

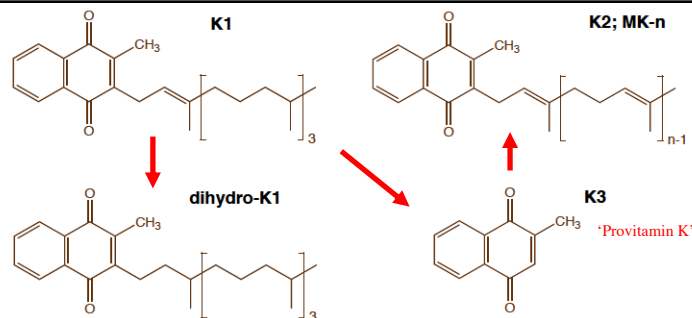
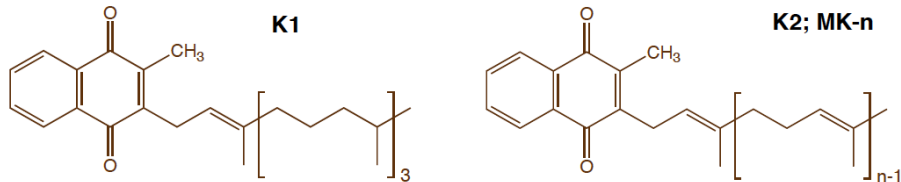
1. Background

Vitamin K is transported in serum to its target tissues via lipoproteins

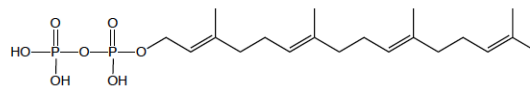
- Vitamin K (the K is for 'Koagulation') was discovered as researchers tried to understand why chickens and other experimental animals fed diets with very low lipid contents developed hemorrhages and why blood taken from these animals clotted slowly.
- In the 1930s, it was found that this hemorrhagic 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

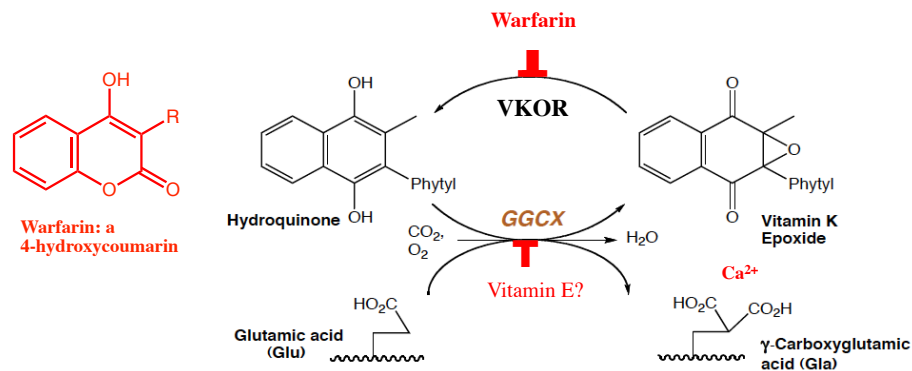
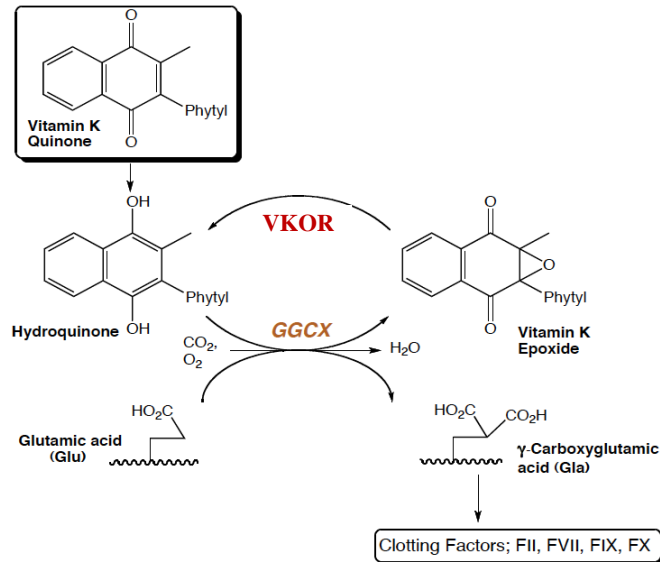
- A group of 3-substituted, 2-methyl-1,4-naphthoquinones having anti-hemorrhagic activity.



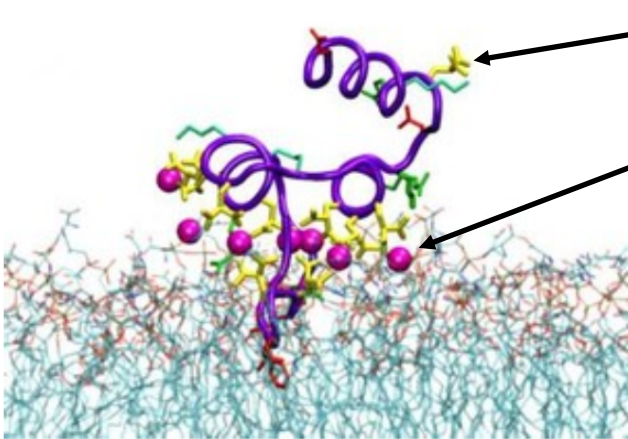
- K1 – Phylloquinone, most prevalent form of vitamin K found at high concentrations in green leafy vegetables.
- 2'-3'-Dihydro-K1 – a form of vitamin K produced during the hydrogenation of vitamin K1-rich vegetable oils.
- K2 – series of Menaquinones, at least 13 known (MK1-MK13).
 - ✓ MK-4, present 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 – Menadiene, '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 γ -carboxylation of glutamic acid residues (an activation process that forms Gla proteins) on several precursor proteins with important biological functions.



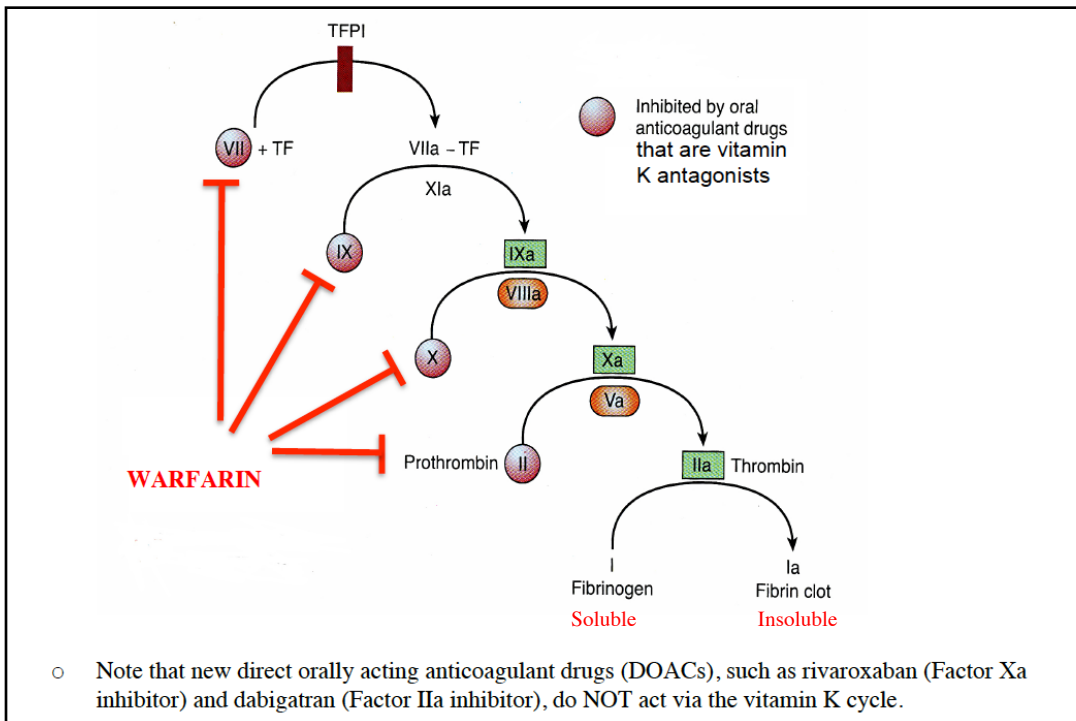
- *VKORC1* is the gene that encodes the enzyme, vitamin K epoxide reductase enzyme (VKOR), which is the target for warfarin and other vitamin K antagonists.
- Warfarin inhibits VKOR, thereby reducing the recycling of reduced vitamin K in the liver.
- GGCX is the gene encoding the γ -glutamyl carboxylase 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.

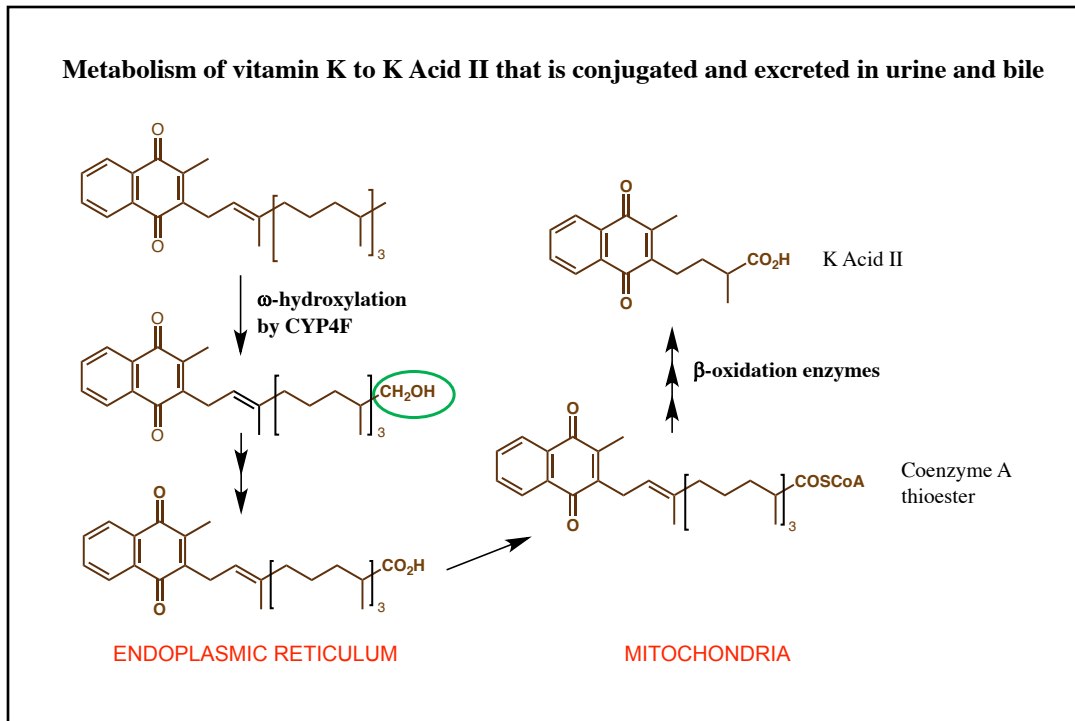


Gla residue

Calcium ion

- 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.





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.
- PIVKA- Protein Induced by Vitamin K Antagonist

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 γ -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 γ -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.

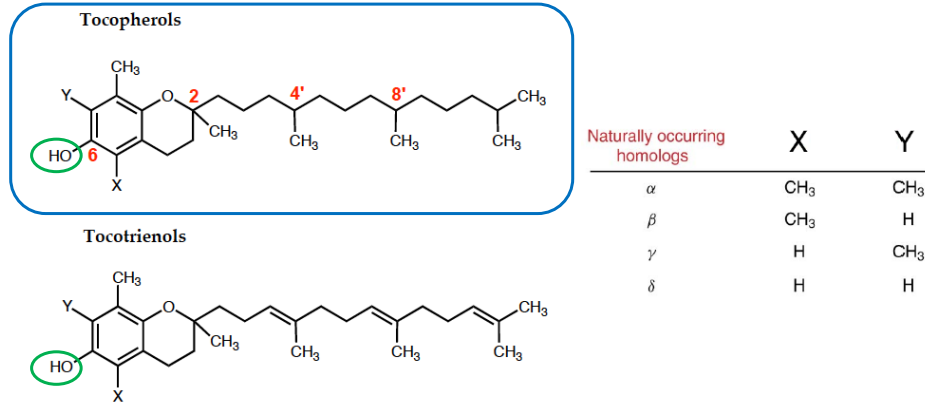
10. Consumer Counseling

- 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')

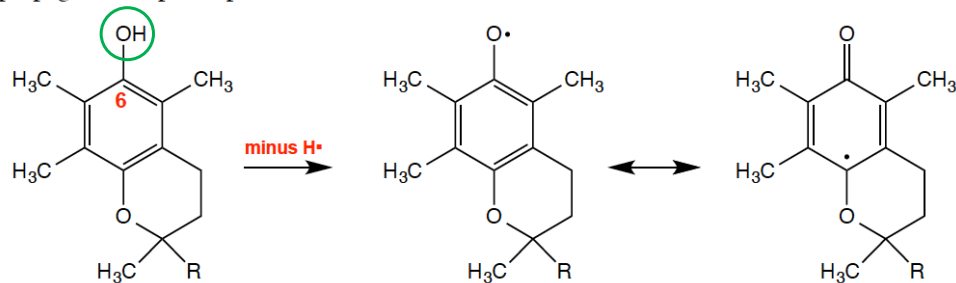
1. Structures

- 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) 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 $2R,4'R,8'R$.



2. Antioxidant properties

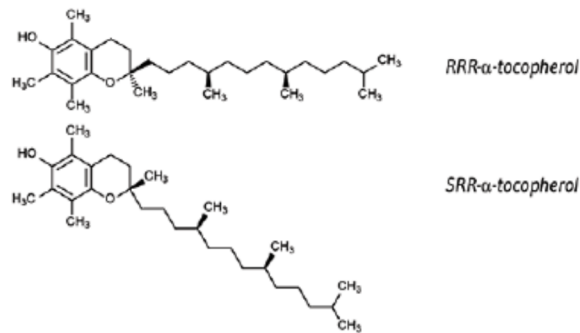
- Vitamin E has an important function as an antioxidant. One electron oxidation of α -tocopherol leads to the resonance stabilized radical shown below. Facile donation of H to peroxy radicals (ROO \cdot) neutralizes them and terminates lipid peroxidation at the propagation step (see p.25 for details).



- 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. α -Tocopherol is the most potent and δ -tocopherol the least potent antioxidant in vitro.

3. Pharmacological activity and transport

- α -Tocopherol is the most important form of vitamin E. As assessed by the rat resorption-gestation test, *RRR*- α -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 β -, γ -, δ -tocopherol nor the tocotrienols contribute to the body's vitamin E requirement because, although absorbed, are poorly recognized by the α -tocopherol transport protein (α TTP) in the liver.



- α TTP is responsible for the selective transfer of (2*R*)- α -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).

- <i>RRR</i> - α -tocopherol	(natural)	1 mg = 1.5 I.U.
dl - <i>all-rac</i> - α -tocopherol	(synthetic)	1 mg = 1.1 I.U.
dl - <i>all-rac</i> - α -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.

Deficiency state (continued)

- 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 α -tocopherol to total plasma lipids (< 0.8 mg/g), or having a plasma level < 20 μ 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 γ -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.

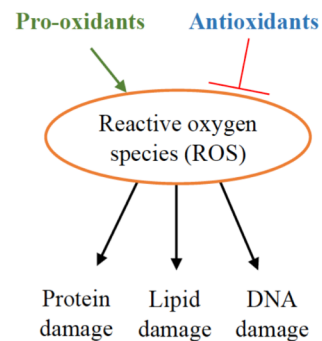
OXIDATIVE STRESS AND PROTECTIVE MECHANISMS THAT INVOLVE VITAMINS (and MINERALS)

Focus here is on:

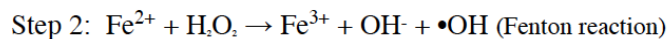
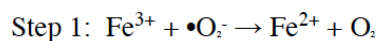
- Minerals like Fe, Cu and Zn, which are important for enzymes that detoxify reactive oxygen species (ROS).
- Vitamins and minerals (Se) in the glutathione pathway that detoxifies (lipid) peroxides
- The synergy between vitamins E and C in scavenging of (lipid) radicals.

Oxidative stress

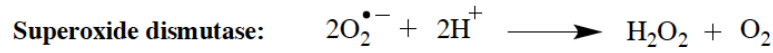
- Oxygen is essential to life, but obscures the fact that it is also a poison and aerobes survive only because they have evolved antioxidant defenses.
- The oxidative status of cells is determined by the balance between *antioxidants* and *pro-oxidants*.



- 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_2^-]
 - ✓ Hydroxyl radical [OH^\cdot]
 - ✓ Hydrogen peroxide [H_2O_2]
 - ✓ Peroxyl radicals [ROO^\cdot]
- The Haber-Weiss reaction generates hydroxyl radical from superoxide and hydrogen peroxide in two steps catalyzed by ferric and then ferrous iron.



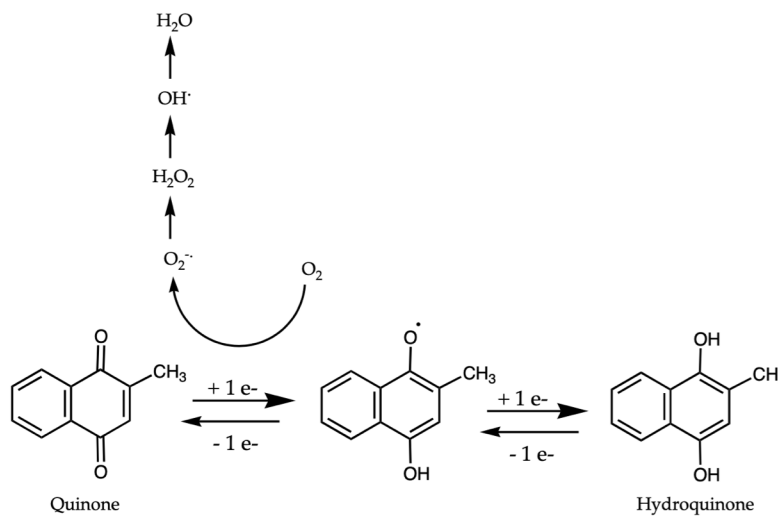
Enzymes that degrade hydrogen peroxide and superoxide



- Cytosolic SOD1 and extracellular SOD3 use **Cu and Zn**, whereas mitochondrial SOD2 has a **Mn** cofactor
- Catalase (peroxisomes) is a heme-containing protein and so needs **Fe**.

Futile Cycling

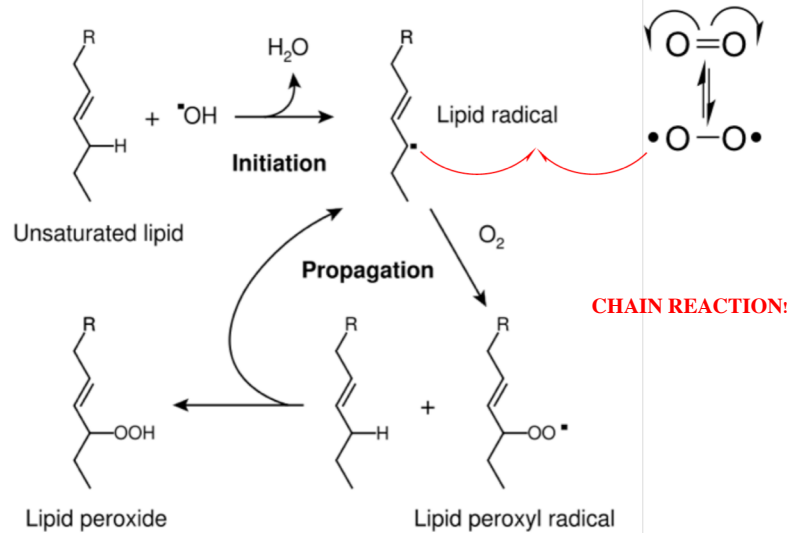
- ROS are also generated during 'futile cycling' of, for example, quinones.
- Sequential additions of single electrons to molecular oxygen generate superoxide anion, hydrogen peroxide and hydroxyl radical.



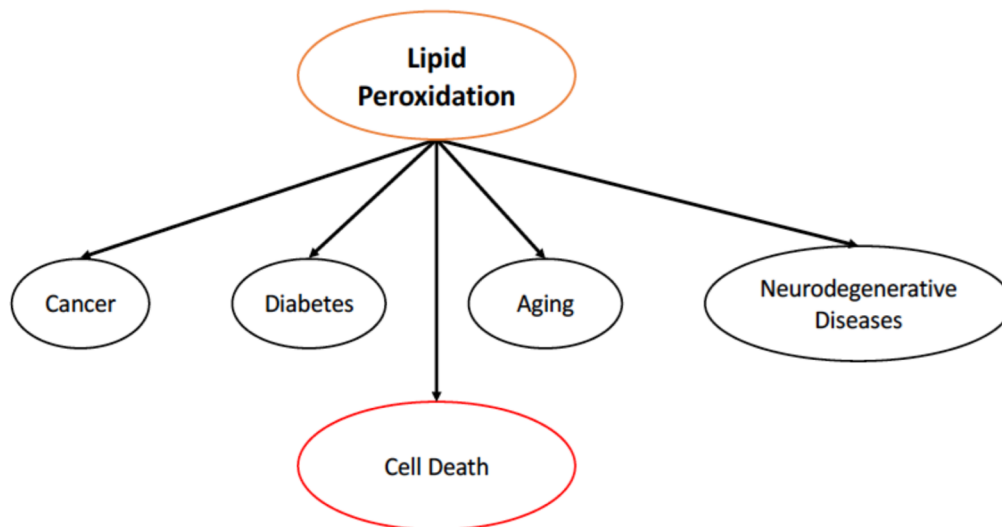
- Other sources of e^- : Reduction of nitroaromatics, mitochondrial respiration, UV light radiation.

Major Targets of ROS - DNA, proteins, lipids, e.g., unsaturated lipid.

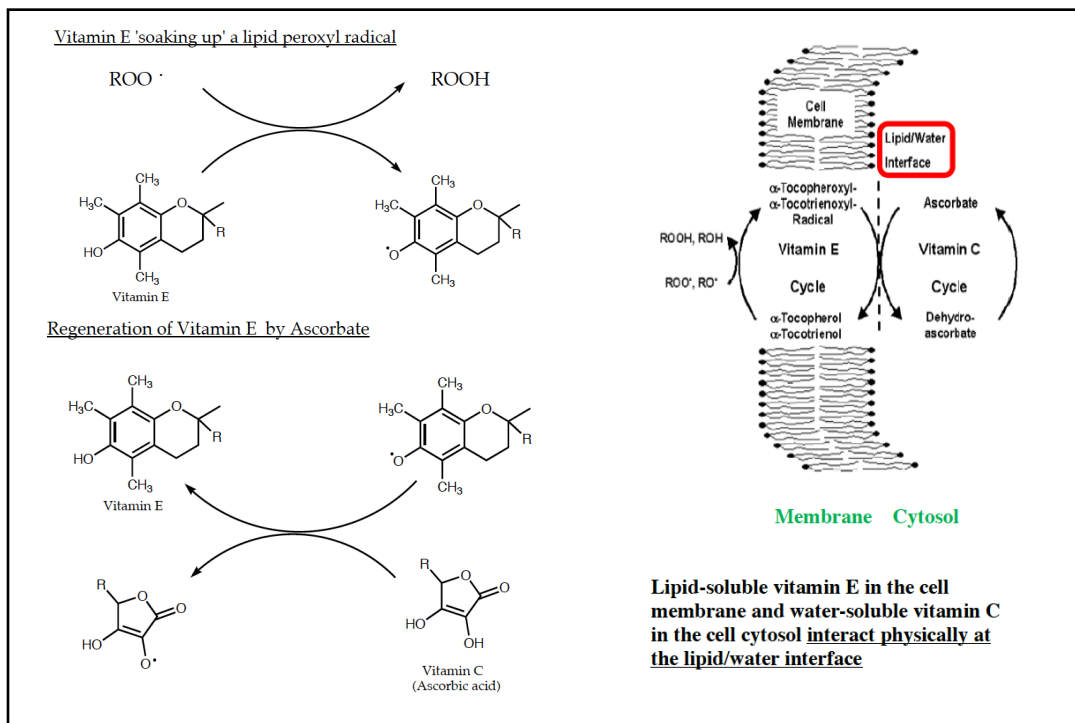
- ROS causes tissue damage through promotion of lipid peroxidation with subsequent damage to biological membranes.



Pathological Effects of Lipid Damage (Peroxidation)



- Lipid peroxidation 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 (ROO[•]) with vitamin E, **instead of another unsaturated lipid molecule**.
- Remember, Vitamin E is the main lipophilic, chain-breaking antioxidant present in cell membranes.

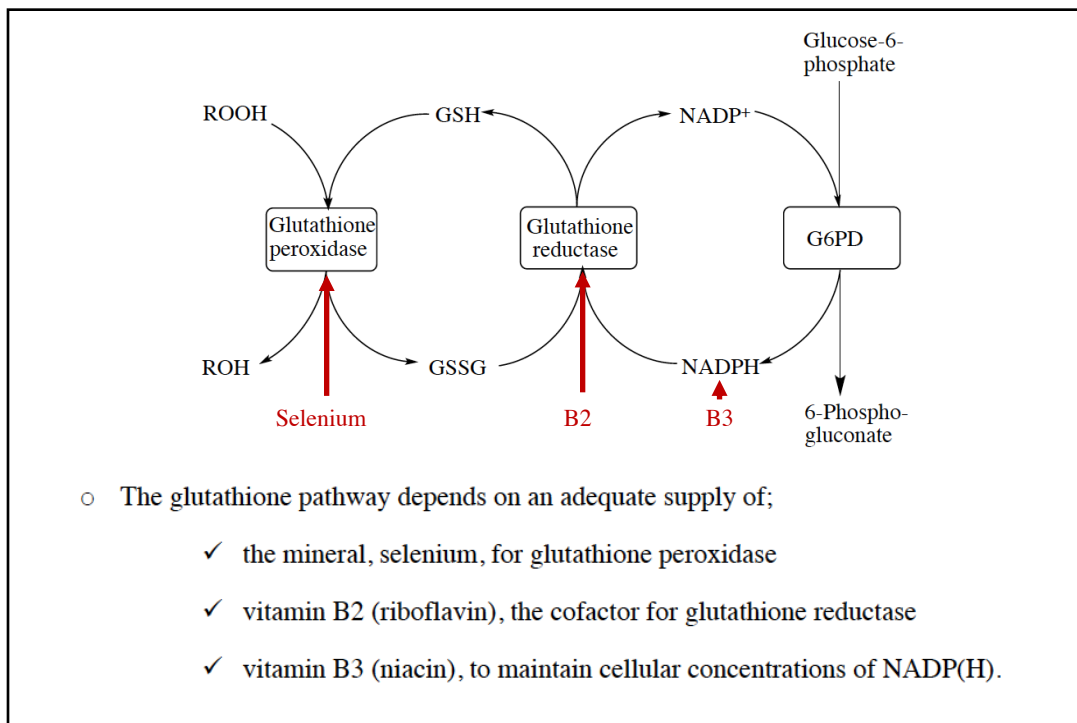
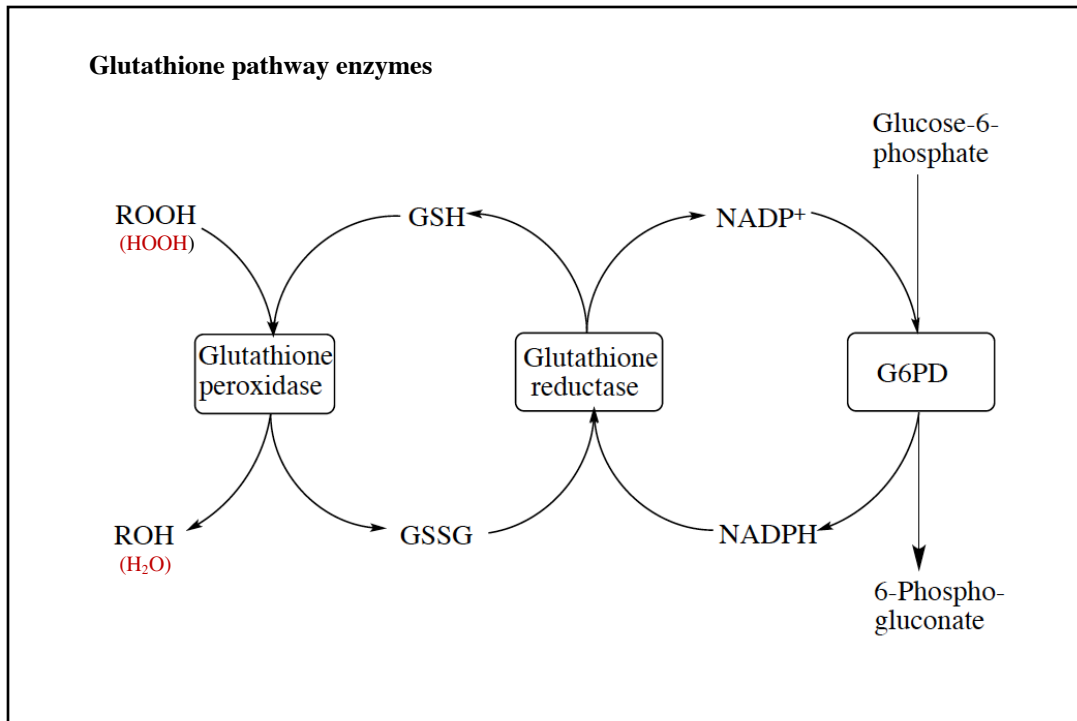


- Note: Vitamin E is NOT a potent scavenger of hydroxyl, nitroxyl or thiyl radicals – ONLY lipid peroxy radicals.
- The antioxidant action of vitamin E is enhanced by vitamin C, which can react with the resulting oxygen-centered vitamin E radical to regenerate vitamin E.
- Antioxidant clinical trials 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.



FAT-SOLUBLE VITAMINS: STUDY SUMMARY

Final on Wednesday Dec 11, total 100 pts

- (Xu 50 pts)

- Rettie 50 pts, multiple choice and short answer.

- **Chemistry/Metabolism - Pathways and Enzymes**
- **Functions/Therapeutic Uses**
- **Deficiency States/Symptoms**
- **Toxicities**
- **Daily Requirements/Upper Limits**

ALL RETTIE NOTES MATERIAL IS FAIR GAME