Part 2: Fat-Soluble Vitamins and Minerals

I. Generalities

• Fat-soluble vitamins – ADEK is the acronym.

  • Vitamers are different forms of a particular vitamin, e.g. vitamins K1 and K2, vitamins D2 and D3, etc.

  • Unlike water-soluble vitamins that need regular replacement in the body, 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 generally act directly (e.g. vitamin E) or bind to specific receptors in the cell nucleus to influence gene expression (e.g. vitamin D, vitamin A).

  • Fat-soluble vitamins are absorbed in association with dietary fat - bile is required. Diseases that impair fat absorption (e.g. ulcerative colitis, Crohn’s) can lead to deficiencies.

  • Fat-soluble vitamins are largely stable to heat/cooking. Several are light sensitive - vitamin A/vitamin E and K.

  • Most people will not need supplementation with fat-soluble vitamins. Only certain at-risk populations, e.g. lactating and post-menopausal women, newborns, alcoholics.
II. Vitamin A

1. Chemistry and Metabolism
   - A series of retinoid and carotenoid compounds containing the β-ionone ring are pharmacologically active. Vitamin A is found in the body as retinol, retinal and retinoic acid.
   - Among the retinoids, retinol (mixed esters, especially retinol palmitate) is mostly what is ingested from animal products.

   ![Chemistry and Metabolism Diagram](image)

   **Figure 1**
   
   (a) The structures of retinoids. (b) The major metabolic pathways of vitamin A (retinol) in mammalian cells. Not all of these enzymes are expressed in all cells. Abbreviations: ADH, retinol dehydrogenase (17, 149); AKR1B1, aldo-keto reductase family 1, member B1 (20, 21); AKR1B10, aldo-keto reductase family 1, member B10 (20, 21); ALDH, retinaldehyde dehydrogenase (149); CYP26, cytochrome P450 family (25); LRAT, lecithin:retinol acyltransferase (11, 12); REH, retinyl ester hydrolase (6, 150); RetSDR, retinol short-chain dehydrogenase/reductase (19).

   - Retinol dehydrogenases (ADH)/aldo-keto reductases (AKR) and retinal dehydrogenases (RALDH) convert retinol \(\leftrightarrow\) retinal \(\rightarrow\) retinoic acid, respectively
   - CYP26 metabolizes retinoic acid by 4-hydroxylation on the β-ionone ring.
   - Trans and cis isomers are pharmacologically active. E.g. 9-cis and 13-cis RA use in cancer/acne.
   - 11-cis retinal and 11-trans retinol undergo cis-trans isomerization catalyzed by light and certain metabolic enzymes, respectively.
Carotenoids are divided into the *carotenes* – which contain no oxygen, and the *xanthophylls* - which do contain oxygen atoms.

- Of the ~600 plant carotenoids identified, only the few that contain an unmodified β-ionone ring have pro-vitamin A activity.

- β-Carotene is the most potent carotenoid because it yields (theoretically) 2 moles of retinal after cleavage by β-carotene 15,15’-dioxygenase. However, the enzymatic process is inefficient and conversion decreases when body stores of vitamin A are high.
2. **Dietary sources** – plants (carrots, sweet potatoes, red peppers) provide carotenoids, animal (livers) provide retinol palmitate/stearate. Fish (liver) oils, eggs and fortified dairy products are also important sources of vitamin A.

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

4. **Functions and deficiency states** - The active forms of vitamin A have 3 basic functions; vision, growth and development, immunity.

**Vision** – vitamin A is an integral part of the rhodopsin visual cycle.

- The rhodopsin cycle involves two critical isomerizations; the first, catalyzed by light, converts 11-cis retinal (bound to opsin) to 11-trans retinal.
- The second, catalyzed by retinoid isomerase, 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 to form the low light sensitive pigment, rhodopsin.

![Rhodopsin Diagram](image)

**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 also 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.
5. **Daily requirement** – Daily value (DV) = 5000 IU. UL = 10,000 IU (~ 3000 µg) -1 µg retinol is about 3.3 IU).

- 1 retinol activity equivalent (RAE) = 1 µg all trans retinol = 2 µg all trans β-carotene in oil (a highly absorbable form) = 12 µg food-based all trans β-carotene = 24 µg other mixed dietary carotenoids. Supplements usually provide the acetate ester.
- Adult DRI = 900 µg retinol equivalents (~ 2900 IU) for males and 700 µg/d (~ 2300 IU) for females and 1300 µg/d (~4000 IU) for breast-feeding mothers.

6. **Uses**

a. Deficiency state is extremely rare in US; 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.

b. Low vitamin A intake is associated with more severe infectious diseases, including HIV.
   - The infectious process also lowers vitamin A.
   - Retinol is being evaluated by the WHO in some developing countries to decrease mortality in children due to measles and other infectious diseases. Vitamin A supplements reduced child mortality by 24% in low- and middle income countries. (BMJ, 2011, 343:d5094. doi: 10.1136/bmj.d5094.

c. Carotenoids have antioxidant/free radical scavenger activity.
   - Lycopene, a carotenoid (diets rich in tomatoes) with no vitamin A activity, may have benefit in preventing prostate cancer; doses of 6-30 mg.
   - Luteine, a carotenoid (diets rich in 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.

d. Cancer - retinoic acid is important for promoting cell differentiation and inhibiting cell proliferation.
   - Low vitamin A is associated with an increased risk of various cancers.
   - Tretinoin (all trans retinoic acid) used in patients with acute promyelocytic leukemia, acts through induction of terminal differentiation.
   - 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 β-carotene to smokers resulted in an increased risk of lung cancer.

e. Skin conditions- preserves epithelial morphology.
   - Acne -- topically as retinoic acid. Systemically as 13-cis retinoic acid (isotretinoin) Accutane® Roche.
   - Psoriasis – etretinate (Tegison®) ↦ acitretin (Soriatane®)
   - **Attention:** these retinoids are strong teratogens.
7. Vitamin A-related drugs

all-trans Retinoic Acid
TRETINOIN

13-cis Retinoic Acid
ISOTRETINOIN

9-cis Retinoic Acid
ALITRETINOIN

ETRETINATE

ACITRETIN

BEXAROTENE
8. **Toxicity**

   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/5 ml. Beef liver has about 30,000 IU/3 oz.
      - Watch out for **polar bear liver** -- has 20,000 to 30,000 I.U./g!!!

   c. **Teratogenic?** This is controversial. Significant increase in the risk of vitamin A-associated birth defects has been observed at (preformed) vitamin A doses above 10,000 I.U./day (Rothmann et al., NEJM, 1995).
      - Others suggest no problem at doses <30,000 IU/day.
      - Concerns have been raised over liver consumption by pregnant women.

   d. **Risk for fractures** – an intake 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.

9. **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.

   c. Avoid β-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 the retinal by the 15,15-dioxygenase 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.
III. Vitamin D

1. Two main forms; vitamin D2 and D3

- Technically, vitamin D is not a vitamin. It is the name given to a group of fat-soluble prohormones (substances that are precursors to hormones, and which usually have little hormonal activity by themselves).

- 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.

- Vitamins D2 and D3 are secosteroids. These are very similar in structure to the normal tetracyclic steroid nucleus, but one of the rings is incomplete.

- Two major form 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.

- Controversy has long existed about the relative potency of D2 versus D3. In humans, a case can be made for using only D3 because:
  - it 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 and Metabolic Activation**

7-Dehydrocholesterol

![Diagram showing the synthesis and metabolic activation of vitamin D3]

- Skin, UV light
- Skin, heat
- Liver, 25-hydroxylase
- Kidney, 1α-hydroxylase
- 24-hydroxylase

**25-OH Vitamin D3**

**Vitamin D3**

**1,25 (OH)2 D3**

**1,25 DHCC**

**Active hormone**

**24,25-(OH)2 D3**

Inactive
Note: Photoactivation of 7-dehydrocholesterol at 295 nm requires sun angle >45° above horizon. 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.
- VDR activation enhances gene expression of Ca\(^{2+}\) binding and transport proteins involved in Ca\(^{2+}\) (and phosphate) absorption in the intestine and in 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 homoestasis.

![Diagram of calcium regulation](image)

- VDR activation in bone modulates bone mineralization.
- In kidney failure, renal synthesis of vit. D3 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**.
  o 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.
  o Laboratory studies have shown that calcitriol promotes cellular differentiation, decreases cancer cell growth and stimulates apoptosis - ‘programmed cell death’.
  o Another possible mechanism involves genetic variation within the vitamin D receptor itself, and several studies have linked the presence of vit. D receptor polymorphisms with cancer development.
  o High profile report (Lappe et al., Am. J Clin. Nut., 2007) found: 1100 IU of vitamin D and 1500 mg of calcium per day administered to 403 Nebraska women over 4 years dramatically reduced the relative risk (0.232) for incident cancers compared with 206 placebo controls (p < 0.005). Furthermore, baseline and treatment-induced serum 25-hydroxy-vitamin D (25[OH]D) levels were strong and independent predictors of cancer risk.
  o 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. **Source** -- fish liver, fish products, sunshine, eggs (in D supplemented hens), liver, milk (fortified). Cod liver oil has about 400 IU/5ml.

5. **Requirements** -- DV = 400 IU; UL = 4000 IU.
   **Note:** In late 2010, IoM increased their daily AI level to 600 IU/day for adults and their UL to 4000 IU (from 2000 IU). 1 µg = 40 IU.

6. **Toxicity**
   o As with Vitamin A, vitamin D overdose typically happens over a period of time rather than from a single large dose.
   o For children under the age of 12 months, a sustained intake of 1,000 micrograms (40,000 IU – 40X the UL!!) a day will produce severe toxicity (i.e. calcification of soft tissues such as the lung, kidney) in one to four months.
   o For adults, 2,500 micrograms (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-D. Natural levels in adults who live or work in the sun are 50-70 ng/ml. Levels needed to prevent rickets and osteomalacia, are 15 ng/ml. Deficiency commonly identified as levels <20 ng/ml.

8. **At risk for deficiency**
   o Infants/Elderly with minimal sun
   o Dark skin with minimal sun
   o Religions that require the entire body be covered
   o Fat malabsorption
   o Inflammatory bowel diseases
   o Kidney failure
   o Seizure disorders treated with anticonvulsants which increase vit. D elimination
9. **Use** – the importance of adequate intake and (perhaps) the value of using supplements of this vitamin is now beginning to be realized.
   
a. Deficiencies due to low sun exposure → osteomalacia and osteoporosis.
   
b. 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.
   
c. 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.

   Use (Rocaltrol® and generic products) to provide this active metabolite directly. Available in capsules and as an oral solution, 0.25 – 0.5 µg.

   Also Paricalcitol (Zemplar®) – Modification of 1,25 DHCC used po for hyperparathyroidism. 1 µg three times per week.

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10. **Consumer Counseling and Advice**

a. Assure intake of at least 400 IU/d. Multivitamins usually contain this amount.

b. 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.

c. Vitamin D is very important for bone health, but also may help reduce risks for cancer and other diseases.

d. Postmenopausal women should take a vitamin D supplement as well as calcium supplement.
IV. **Vitamin K** - Group of 3-substituted, 2-methyl-1,4-naphthoquinones having anti-hemorrhagic activity.

1. **Structures**

   - **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, formed from K3 in the body, via a complex, poorly understood reaction.
     - MK-7, high concentrations in some fermented foods, e.g. natto.
     - MK-8-13, synthesized by bacteria in the gut.
   - **K3** – Menadione, ‘provitamin’, lacks side-chain that is required for vitamin K activity.

2. **Function** – Vitamin K cycle activity, necessary for carboxylation of γ-glutamyl residues (Glu→Gla modification) on precursor proteins.

   - Ca$^{2+}$ ions bind to Gla residue clusters at the N-terminus of the protein, inducing a conformational change that forms a hydrophobic patch that mediates Gla protein interactions with phospholipids on the cell surface membrane.
2. **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 that **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.

3. **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.
     - The neonatal liver is immature with respect to (descarboxy) prothrombin synthesis.
     - 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 25mg/d for 2 years decreased hip fractures in an older population, but studies are inconclusive about benefit.
   - Cancer – Emerging role for K2 in particular in diagnosis/prevention of hepatocellular carcinoma.
   - Possible therapeutic role for vitamin K2 in preventing vascular calcification?

5. **Source** – Green leafy vegetables; esp. spinach, collard greens, kale, parsley, broccoli.

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

7. **Toxicity**– Some allergic reactions reported IV, otherwise nothing special.

8. **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 and avoid fluctuations in warfarin dose.
V. Vitamin E

1. Structures
   - The term Vitamin E is used for a family of 8 different molecules; four tocopherols and four tocotrienols.
   - 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. Vitamer activity, transport and metabolism
   - \( \alpha \)-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 \( 2R \)-configuration are considered to contribute to satisfying vitamin E requirements in humans.
   - Neither \( \beta \), \( \gamma \), \( \delta \)-tocopherol nor the tocotrienols contribute to meeting the body’s vitamin E requirement because, although absorbed, they are recognized poorly by the \( \alpha \)-tocopherol transport protein in the liver. This transporter is responsible for the selective transfer of \( (2R)\)-\( \alpha \)-tocopherol into VLDL (with subsequent distribution to other serum lipoproteins).
   - \( RRR-\alpha \)-tocopherol 1 mg = 1.5 I.U.  natural
   - \( all-rac-\alpha \)-tocopherol 1 mg = 1.1 I.U.  synthetic
   - \( all-rac-\alpha \)-tocopherol acetate 1 mg = 1.0 I.U.  synthetic
   - Vitamin E metabolism is initiated by CYP4F2 to the \( \omega \)-hydroxy metabolite which is then further oxidized to the carboxylic acid that serves as a substrate for \( \beta \)-oxidation to chain-shortened acid metabolites that are excreted in urine.
3. 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 that reacts readily with lipid peroxyl radicals (ROO*) to neutralize them.

\[
\begin{align*}
\text{OH} & \quad \rightarrow \quad \text{O} \cdot \\
\text{H}_3\text{C} & \quad \text{H}_3\text{C} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{H}_3\text{C} & \quad \text{H}_3\text{C} \\
\text{R} & \quad \text{R}
\end{align*}
\]

- 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 (see p.21 for details).
- The antioxidant potency depends on the substitution pattern of the methyl groups on the aromatic ring.

<table>
<thead>
<tr>
<th>tocopherol/ tocoptrieno</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>relative antioxidant activity (%) in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
<td>100</td>
</tr>
<tr>
<td>β-</td>
<td>CH$_3$</td>
<td>H</td>
<td>71</td>
</tr>
<tr>
<td>γ-</td>
<td>H</td>
<td>CH$_3$</td>
<td>68</td>
</tr>
<tr>
<td>δ-</td>
<td>H</td>
<td>H</td>
<td>28</td>
</tr>
</tbody>
</table>

4. 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.

5. Deficiency state
- ‘Tokos’ is Greek for birth. Deficiency in rats causes sterility in male rats and fetal resorption in pregnant females.
- In humans, deficiency is generally characterized by neuromuscular abnormalities and myopathies. These peripheral neuropathies are considered to be due to free radical damage to nerves.
- Rare (in developed countries), usually due to fat malabsorption.
- Patients with rare genetic defects in α-tocopherol transport protein are vitamin E deficient and also present with peripheral neuropathy, in the absence of fat malabsorption.
- A deficiency state (often characterized by hemolytic anemia, fragile RBCs damaged by free radicals) has been seen in some premature infants where stores of vitamin E are low at birth due to poor placental transport.
- Diagnosis is based on measuring the ratio of plasma α-tocopherol to total plasma lipids (< 0.8 mg/g).
6. **Daily requirement** -- DV = 30 I.U (20 mg natural, 30 mg synthetic). UL = 1000 mg (1500 IU natural or 1000 IU of synthetic vitamin E acetate).

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.
   - Mechanism has been suggested to involve inhibition of the $\gamma$-carboxylase enzyme in the vitamin K cycle by vitamin E quinone. [Direct effect on platelet function may also contribute].

8. **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.
   - Heart Disease- No benefit in dozens of trials comparing risk of cardiovascular death or atherosclerosis disease progression. *Am. J Cardiol.* 2008 101(10A) 14D-19D.
   - All cause mortality – A small increase at doses over 400 IU. Owever, most studies were in high risk populations. A small decrease in mortality in doses <400 IU (Ann Intern Med. 2005 Jan 4;142(1):I40.)
   - Alzheimers Disease -- high doses (2000 IU/d) showed some benefit in slowing progression, but not in prevention.
   - Retrolental fibroplasia and brochopulmonary dyspasia. Eye and lung damage in premature infants on oxygen. I.V. vitamin E (MVI Pediatric, Astra] seems to offer some protection.

9. **Consumer Counseling and Advice**
   - Based on recent evidence, supplements higher than 200 IU are not beneficial and could be harmful.
   - The amount in a multivitamin is probably adequate (30 IU) for most.
   - Natural vitamin E (RRR) is better utilized than the synthetic racemate.
   - The hoped for substantial health benefits from high dose vitamin E supplements does not seem to have materialized.
   - Use vitamin E supplementation cautiously if there is any tendency to bleed easily.
VI. OXIDATIVE STRESS AND PROTECTIVE MECHANISMS THAT INVOLVE VITAMINS (and MINERALS)

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 reactive oxygen species (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/RNS include:
  - Superoxide anion, $O_2^{-}$
  - Hydroxyl radical, $OH^-$
  - Nitric oxide, $NO^-$
  - Hydrogen peroxide, $H_2O_2$
  - Peroxynitrite, $ONOO^-$

  

\[
\begin{align*}
H_2O_2 & \to H_2O + O_2 \\
2Fe^{3+} + O_2^{-} & \to 2Fe^{2+} + O_2 \\
Fe^{2+} + H_2O_2 & \to Fe^{3+} + OH^- + OH^{-}
\end{align*}
\]

\[
\begin{align*}
\text{Quinone} & \quad \overset{+1e^-}{\underset{-1e^-}{\rightleftharpoons}} \quad \text{Hydroquinone} \\
\end{align*}
\]

• Origin of $e^-$: mitochondrial respiration, UV light radiation, oxidation of hydroquinones, flavins, thiols, reduction of nitroaromatics.

• 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 (futile cycling of quinone, nitroaromatics).
**Targets of ROS** - DNA, thiols, enzymes, membranes, collagen, lipids, e.g., unsaturated lipid.

- Lipid peroxidation, a well-known example of oxidative damage to cell membranes and other lipoprotein structures 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 peroxyl radical (ROO·) with vitamin E, the main lipophilic, chain-breaking antioxidant present in cell membrane.

- 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.
Other Protective Mechanisms

1. **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 a 2-electron pathway.
   
   ![Glutathione Pathway Diagram]

   - G-6-P dehydrogenase (G6PDH) is a key enzyme controlling reducing power in cells. It 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).

2. **Superoxide dismutase:**

   \[ 2 \text{O}_2^- \xrightarrow{H^+} \text{H}_2\text{O}_2 + \text{O}_2 \]

3. **Catalase:**

   \[ 2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \]

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

- Should supplements of antioxidant vitamins be routinely recommended?
- Is there evidence that antioxidant use has long-term benefits?
- Are there adverse consequences of taking antioxidant supplements?
- What doses should be used if use is deemed safe and worthwhile?
- Definitive answers to these questions are not currently available.
- Antioxidant clinical trials involving vitamin E have been very disappointing and may have failed for several reasons;
  - inadequate monitoring of vitamin intake,
  - lack of inclusion of vitamin C,
  - short duration, etc.

VII. MULTIVITAMINS

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

B. Cases where multivitamin supplements are worthwhile:

- Inadequate intake -- alcoholics, poor, elderly, dieters, poor diet
- Increased needs -- pregnancy, lactation, infants, smokers, injury, trauma, surgery, infection
- Poor absorption -- elderly, GI disorders, cystic fibrosis, diarrhea, iatrogenic vitamin deficiencies – e.g. long term antibiotic use, cholestyramine