MEDCH 562P

Vitamin D

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 19th century, up to 90% of children in industrialized Western Europe had rickets, and it became abundantly became 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 prohormones (substances that are precursors to hormones, and which usually have little hormonal activity by themselves), i.e. vitamins D2 and D3 are prohormones 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 cellular processes, neuromuscular function and bone ossification.
2. Two main forms; vitamin D2 and D3

- 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:
  - 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.
3. **Synthesis, Metabolic Activation and Inactivation**

![Chemical reactions and pathways involving vitamin D3 metabolism.](attachment:image.png)

- **7-Dehydrocholesterol** is converted to **Pre-Vitamin D3** in the skin under UV light.
- **Vitamin D3** is synthesized from **Pre-Vitamin D3** in the skin under heat.
- **LIVER 25-Hydroxylase** (CYP2R1) converts **Vitamin D3** to **25-OH D3**.
- **KIDNEY 1α-Hydroxylase** (CYP27B1) converts **25-OH D3** to **1,25(OH)2 D3**, the active hormone.
- **Calcitroic acid** is converted to **1,25(OH)2 D3** by **CYP24A1**.
- **1,23,25(OH)3 D3** and **1,25(OH)2 D3 Glucuronide** are formed by **CYP3A4**, **CYP24A1**, and **UGT1A4**.
Photoactivation of 7-dehydrocholesterol at 295 nm

- Requires sun angle >45° above horizon. Almost never happens at high latitudes.

Vitamin D Receptor Activation

• 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).
3. **Function**

- VDR activation enhances gene expression of Ca\textsuperscript{2+} binding and transport proteins involved in Ca\textsuperscript{2+} (and phosphate) absorption in the intestine and in Ca\textsuperscript{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’.
- 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.
- 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-OH D3 levels were strong and independent predictors of cancer risk.
- 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. 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 mg = 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 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.
   - 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 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 1,25DHC elimination by CYP3A4 pathways.

9. **Use** – 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 po 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**

- 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 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.

<table>
<thead>
<tr>
<th>Function</th>
<th>Name of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemostasis (procoagulant activity)</td>
<td>Prothrombin, factors VII, IX, and X</td>
</tr>
<tr>
<td>Haemostasis (anticoagulant activity)</td>
<td>Proteins C, S, and Z</td>
</tr>
<tr>
<td>Artery calcification inhibition</td>
<td>Matrix Gla-Protein (MGP)</td>
</tr>
<tr>
<td>Bone metabolism</td>
<td>Osteocalcin</td>
</tr>
<tr>
<td>Cell growth regulation</td>
<td>Growth-arrest sequence 6 protein (Gas6)</td>
</tr>
<tr>
<td>Functions unknown</td>
<td>Gla-rich protein (GRP)</td>
</tr>
<tr>
<td></td>
<td>Periostin</td>
</tr>
<tr>
<td></td>
<td>Periostin-like factor</td>
</tr>
<tr>
<td></td>
<td>Four transmembrane Gla-proteins</td>
</tr>
</tbody>
</table>
Vitamin K Cycle, Gla protein formation and Blood Clotting

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

3. **Metabolism** – initiated by P450-mediated \(\omega\)-hydroxylation, with subsequent \(\beta\)-oxidation.
4. 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 desacarboxy prothrombin.

4. 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 (desacarboxy) 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 γ-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.
   - Prevention of vascular calcification – possible emerging role related to γ-carboxylation of MGP, the body’s natural calcification inhibitor.


7. 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.

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

9. 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.
Vitamin E

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 $\alpha$-tocopherol leads to the resonance stabilized radical shown below. Facile donation of H' to peroxyl radicals (ROO') neutralizes them and terminates lipid peroxidation at the propagation step (see p.23 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.

<table>
<thead>
<tr>
<th>Tocopherol/Tocotrienol</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>Relative antioxidant activity (%) in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
<td>100</td>
</tr>
<tr>
<td>$\beta$</td>
<td>CH$_3$</td>
<td>H</td>
<td>71</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>H</td>
<td>CH$_3$</td>
<td>68</td>
</tr>
<tr>
<td>$\delta$</td>
<td>H</td>
<td>H</td>
<td>28</td>
</tr>
</tbody>
</table>
3. Pharmacological activity, transport and metabolism

- 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 2R-configuration are considered to contribute to satisfying vitamin E requirements in humans.
- Neither β-, γ-, δ-tocopherol nor the tocotrienols contribute to meeting the body’s vitamin E requirement because, although absorbed, are poorly recognized by the α-tocopherol transport protein in the liver. This transporter is responsible for the selective transfer of (2R)-α-tocopherol into VLDL (with subsequent distribution to other serum lipoproteins). Other vitamin E forms are also better metabolized, so are not conserved in the body.

- RRR-α-tocopherol (natural) 1 mg = 1.5 I.U.
- all-rac-α-tocopherol (synthetic) 1 mg = 1.1 I.U.
- all-rac-α-tocopherol acetate (synthetic) 1 mg = 1.0 I.U.

- Vitamin E catabolism is initiated by CYP4F2 to the ω-hydroxy metabolite which is then further oxidized to the carboxylic acid that serves as a substrate for β-oxidation to chain-shortened acid metabolites that are excreted in urine.

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

- 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 humans, deficiency is generally characterized by neuromuscular abnormalities and myopathies. These peripheral neuropathies are considered to be due to free radical damage to nerves.
- 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 either on measuring the ratio of plasma α-tocopherol to total plasma lipids (< 0.8 mg/g), or having a plasma level <20 µM.

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 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 – 600IU every other day provided no overall benefit in cancer risk among healthy women (JAMA, 2005:294:56).
   - 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).*
   - 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.
   - Possible that negative trial outcomes reflect administered doses of vitamin E that are much too low to have a useful antioxidant effect in vivo *(http://lpi.oregonstate.edu/ss08/vitamine.html).*

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.
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, $ONOO^-$

```
H_2O  
|     
OH^-  
|     
H_2O_2  
|     
O_2^-  
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```
Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2
```

```
Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^-
```

```
Quinone  
\overset{+1\text{e}^-}{\rightarrow}  
\overset{-1\text{e}^-}{\leftarrow}  
OH  
```

```
Hydroquinone
```

o Origin of e\textsuperscript{−}: mitochondrial respiration, UV light radiation, oxidation of hydroquinones, flavins and thiols, reduction of nitroaromatics.

o 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).

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

- 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 peroxyl radical (ROO·) with vitamin E, the main lipophilic, chain-breaking antioxidant present in cell membrane.

Vitamin E 'soaking up' a lipid peroxyl radical

\[
\text{ROO}^· \rightarrow \text{ROOH}
\]

Vitamin E

Reformation of Vitamin E by Ascorbate

\[
\text{Ascorbic acid} \rightarrow \text{Vitamin E}
\]

Vitamin C

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:

- inadequate monitoring of vitamin intake,
- too low a dose, too short a duration
- lack of inclusion of vitamin C
Other Protective Mechanisms

1. **Superoxide dismutase:**
   \[
   2 \text{O}_2^-> \overset{H^+}{\rightarrow} \text{H}_2\text{O}_2 + \text{O}_2
   \]

2. **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 Fe.

3. **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.

   \[
   \begin{align*}
   \text{ROOH} & \rightarrow \text{ROH} + \text{H}_2\text{O} \\
   \text{GLUTATHIONE PEROXIDASE (Selenium)} & \\
   2\text{GSH} & \rightarrow \text{GS-SG} \\
   \text{GLUTATHIONE REDUCTASE (FAD)} & \\
   \text{H}^+ \\
   \text{NADP}^+ & \rightarrow \text{NADPH} \\
   \text{G-6-P DEHYDROGENASE} & \\
   \end{align*}
   \]
   - 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).