

# MEDCHEM562 2019

## Fat-Soluble Vitamins (and Minerals)

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Rautaiainen et al; Dietary supplements and disease prevention – a global overview. *Nature Reviews Endocrinology*, 12:407-420 (2016).



# **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**

## 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 (dry eye) → chronic conjunctivitis → corneal ulceration → blindness
  - ✓ Rickets
- Second phase of interest in vitamin D in the 1980s when the ‘vitamin D cancer hypothesis’ emerged. Colon cancer mortality found to be inversely associated with exposure to UV-B.
- 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 the 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 – bile acid sequestrant, reduces blood levels of cholesterol
  - ✓ Mineral oil - laxative
- Fat-soluble vitamins are largely stable to heat/cooking. Mild heat is OK, frying less so.
- Several are light sensitive - vitamin A/vitamins E and K.

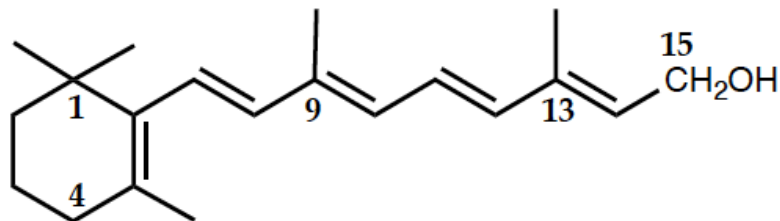
# VITAMIN A

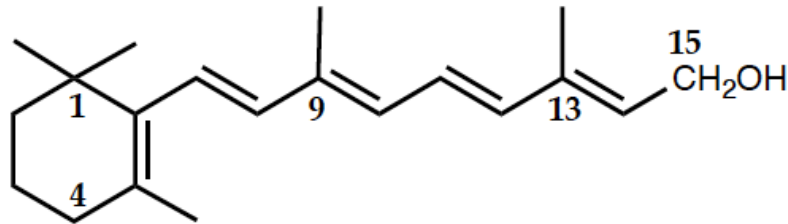
## 1. Background

- 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

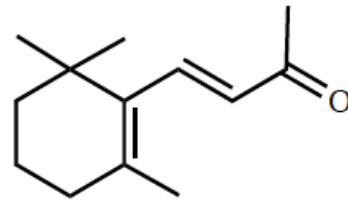
- Vitamin A is a generic descriptor for a series of **retinoid** and **carotenoid** compounds that exhibit the qualitative biological activity of retinol.



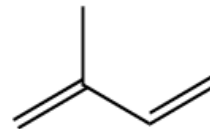


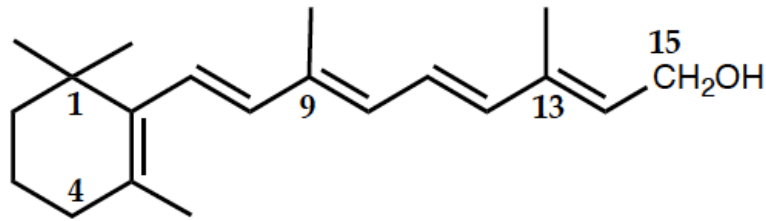
Essential structural features of naturally occurring [preformed] vitamin A (retinol, retinal, retinoic acid)

- Substituted  $\beta$ -ionone nucleus.

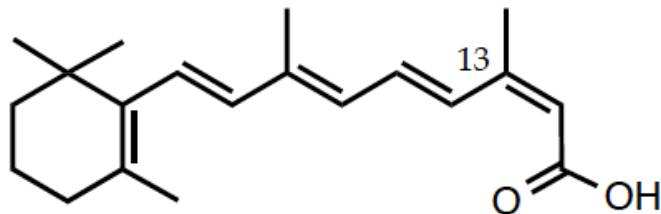


- Polar functional group at C-15 - alcohol, aldehyde or acid.
- Esterification occurs at C-15. Mixed esters, especially retinyl palmitate are the principal storage form and what is mostly ingested from animal sources.
- Side-chain at C-6 composed of isoprenoid units.



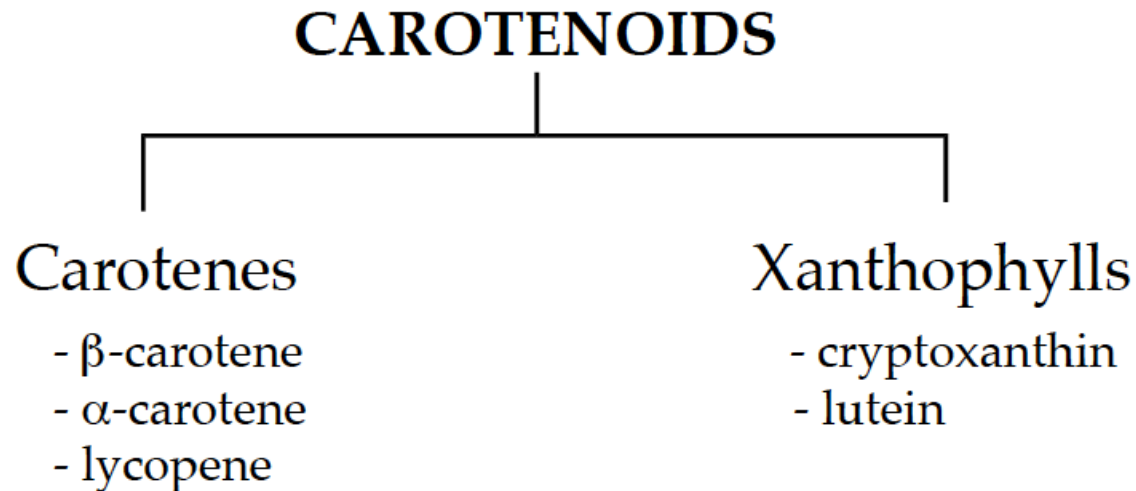


- Conjugated double-bond system extending from C-5. Retinol  $\lambda_{\text{max}} = 325\text{nm}$ , retinoic acid  $\lambda_{\text{max}} = 350\text{nm}$ .
- Many stereoisomers (*cis/trans*) possible, but most of the *cis* isomers are sterically hindered. Important exceptions are 11-*cis* retinal, and 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.



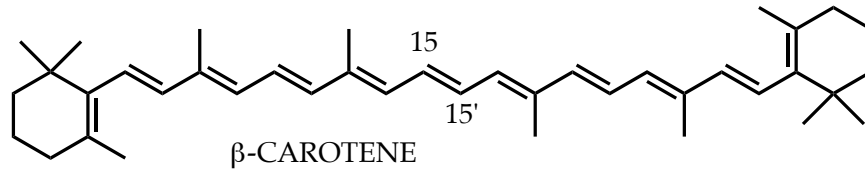
## Carotenoids: Pro-Vitamins A

- 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.



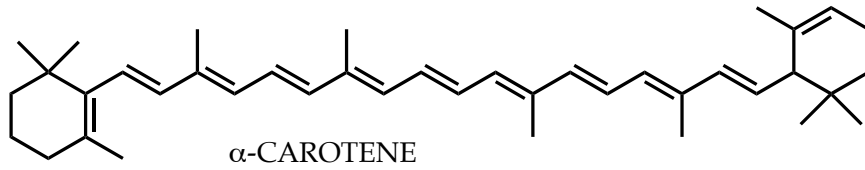
- AREDS (Age-Related Eye Disease Study, 2001) showed lowered risk of progression of AMD (Acute Macular Degeneration) with increased intake of  $\beta$ -carotene and zinc.



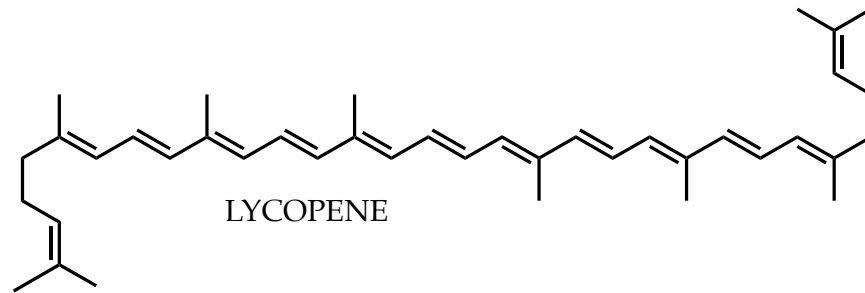


Retinal equivalents (max) ex BCMO

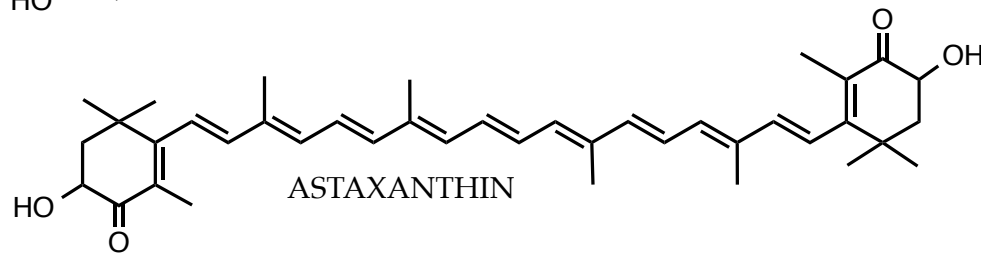
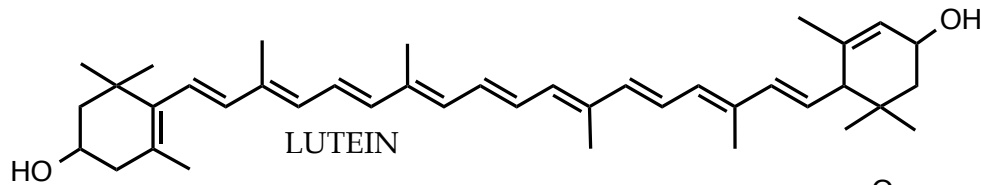
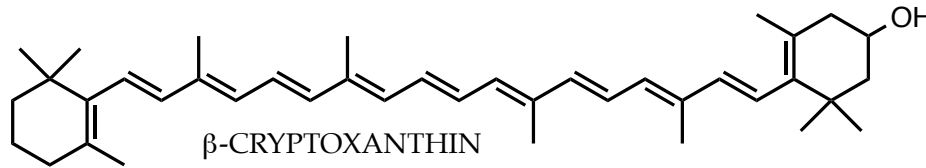
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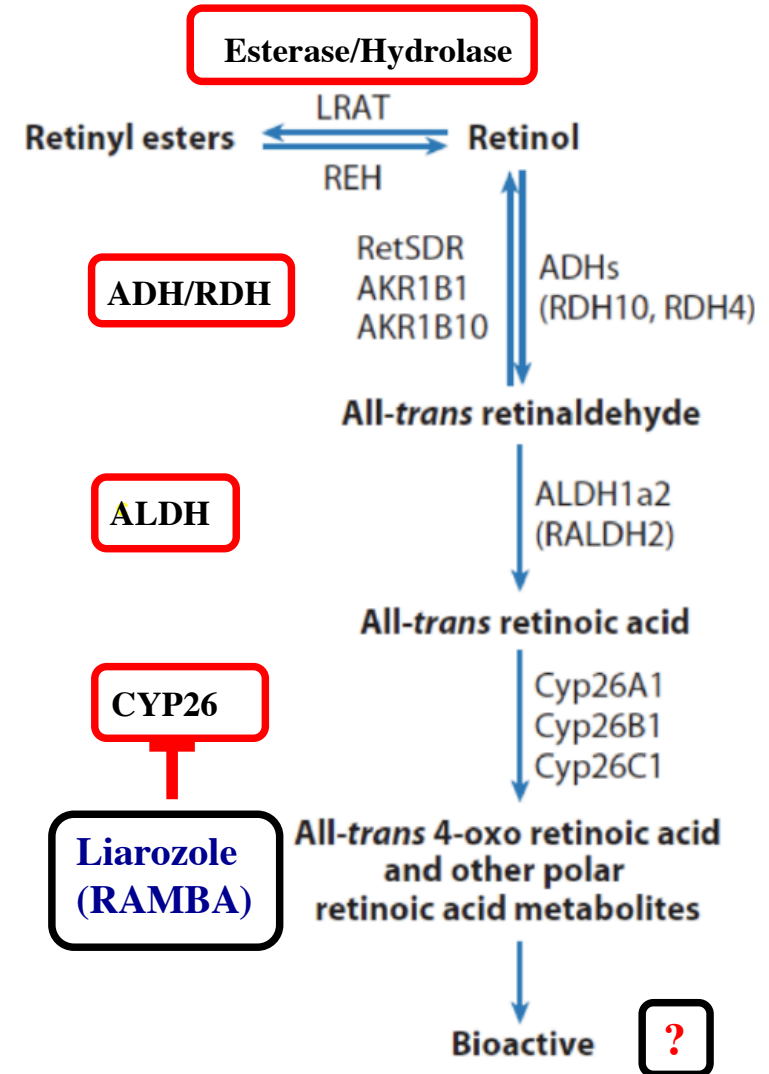
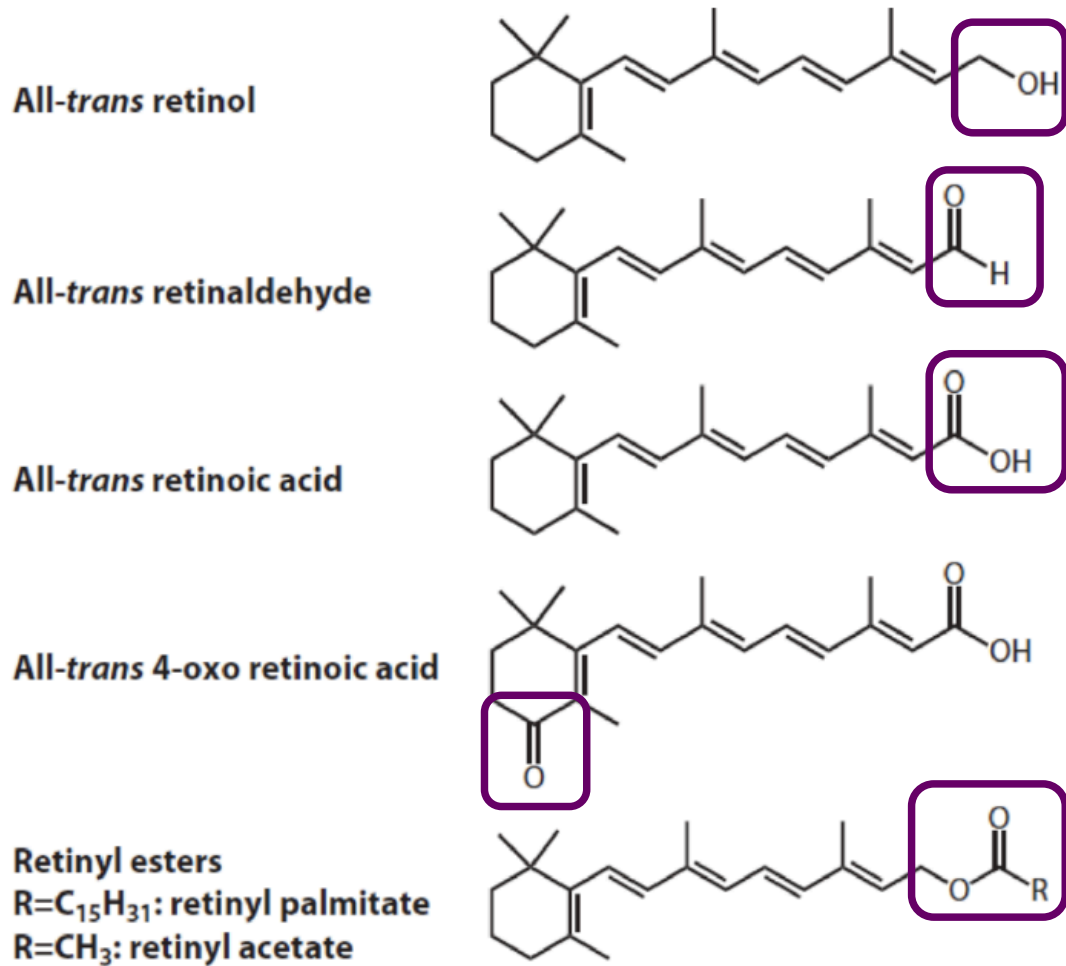
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# Metabolism of Vitamin A



### 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

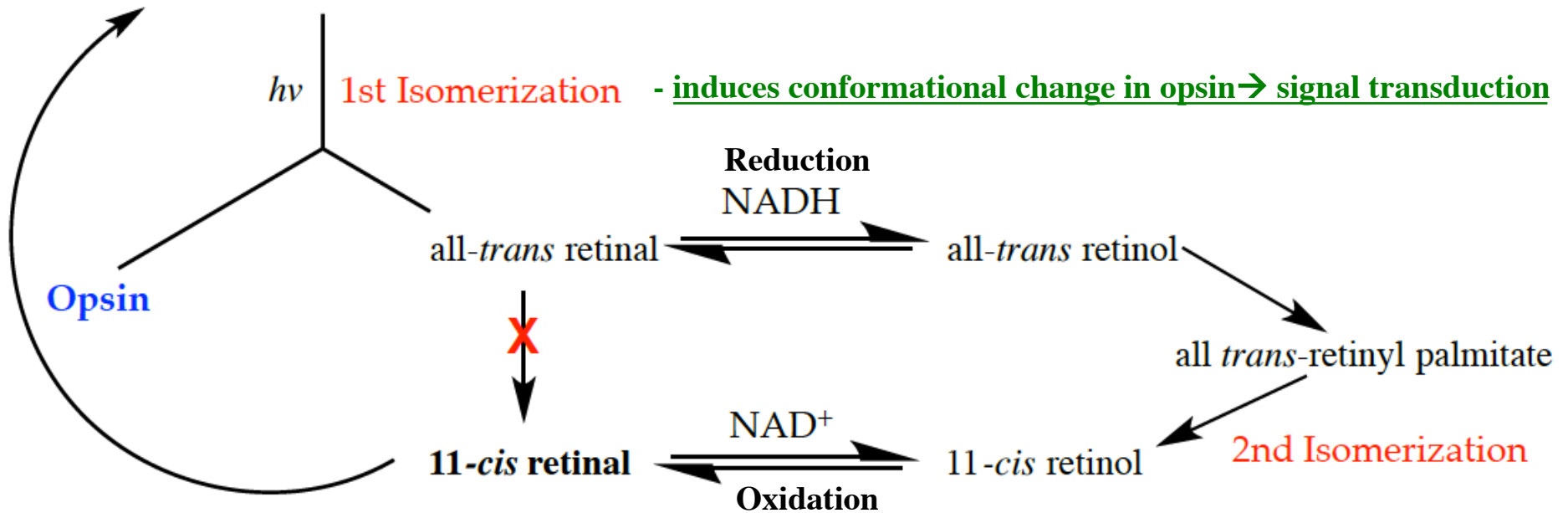
- Specific transport proteins (CRBP, CRABP, RBP) exist for retinoic acid, retinal and retinol.
- Stored in liver as retinol esters (mostly retinyl palmitate).

### 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.

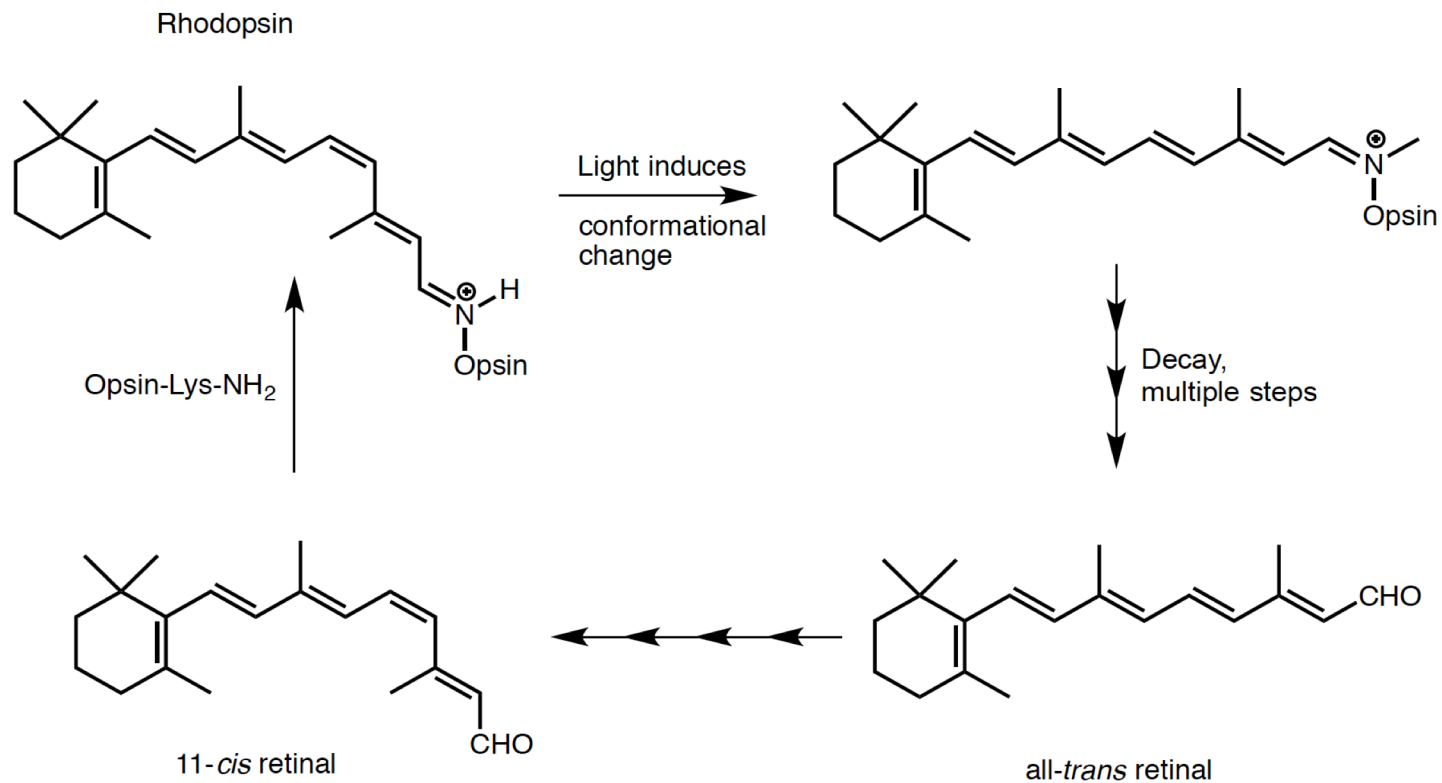
- The rhodopsin cycle involves two critical isomerizations; the first, catalyzed by light, converts 11-*cis* retinal (bound to opsin) to all-*trans* retinal.

**Rhodopsin** (Opsin + 11-*cis* retinal)



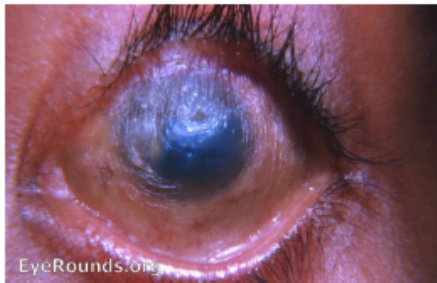
- The second, catalyzed by retinoid isomerohydrolase, converts *all trans* retinyl palmitate to 11-*cis* retinol.
- Oxidation of 11-*cis* retinol generates 11-*cis* retinal, which binds reversibly to opsin *via* a Schiff base with Lys296 (imine formation) to generate the low light sensitive pigment,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

- 11-*cis* retinal binds to opsin and holds rhodopsin – a GPCR - in an inactive conformation.
- When light strikes rhodopsin, isomerization of 11-*cis* retinal to all-*trans* retinal pushes rhodopsin into an active conformation and initiates phototransduction.
- After several decay steps, all-*trans* retinal is released.
- So, opsin must bind new 11-*cis* retinal to continue detecting light.

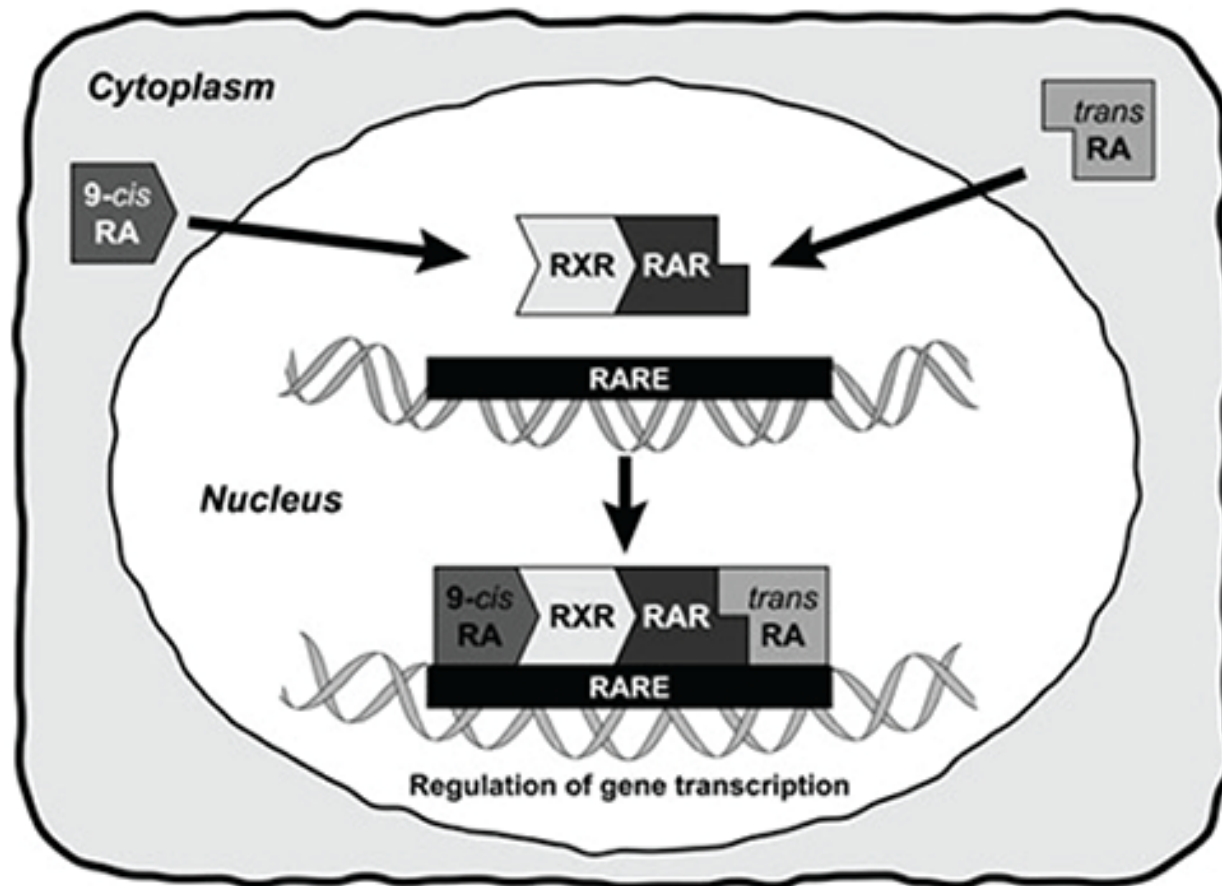


## Growth, Development and Immunity

- Much can be explained by the ability of vitamin A to regulate cell synthesis of macromolecules (*via* RAR and RXR receptor activated transcription).
- Anemia - vitamin A is involved in synthesis of transferrin; deficiency causes low erythrocyte iron that results in anemia.
- Skin - Deficiency here results in low mucin synthesis and high keratin synthesis (hyperkeratosis).
- Keratinization of the cornea results in *xerophthalmia* and risk of blindness, especially in children.



- Skin fissures allow microbe penetration and infection. Vitamin A is known as the “anti-infective vitamin”.
- 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.



All-*trans*-RA and 9-*cis*-RA are transported to the nucleus of the cell bound to cytoplasmic retinoic acid-binding proteins. Within the nucleus, all-*trans*-RA binds to retinoic acid receptors (RAR) and 9-*cis*-RA binds to retinoid receptors (RXR). RAR and RXR form RAR/RXR heterodimers, which bind to regulatory regions of the chromosome called retinoic acid response elements (RARE). Binding of all-*trans*-RA and 9-*cis*-RA to RAR and RXR respectively allows the complex to regulate the rate of gene transcription.



6. **Deficiency** – characterized by plasma retinol  $< 0.7 \mu\text{M}$ .

- 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.
- Vitamin A supplements have reduced child mortality by 20-30% in some low- and middle-income countries (BMJ, Aug 25, 2011, 343:d5094.)

7. **Daily requirement**

- Daily value (DV) = 5000 IU. UL = 10,000 IU ( $\sim 3000 \mu\text{g}$ ) *i.e.* 1  $\mu\text{g}$  retinol is about 3.3 IU). Lowest ratio of UL:DV among fat soluble vitamins. Supplements usually provide vitamin A as the acetate ester.
- 1 retinol activity equivalent (RAE)
  - = 1  $\mu\text{g}$  *all trans* retinol
  - = 2  $\mu\text{g}$  *all trans*  $\beta$ -carotene in oil (a highly absorbable form) - due to low BCMO efficiency
  - = 12  $\mu\text{g}$  food-based *all trans*  $\beta$ -carotene – due to poor absorption
  - = 24  $\mu\text{g}$  other mixed dietary carotenoids – most of which yield 1 mol retinol, not 2 mol



## 8. Uses

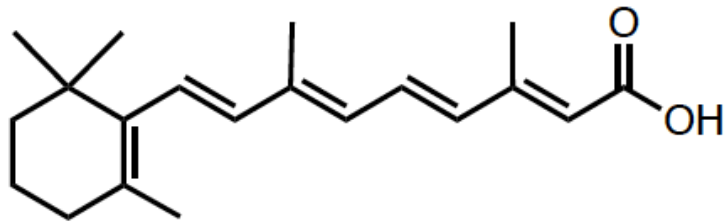
- a. Carotenoids have antioxidant/free radical scavenger activity.
  - Lycopene, a carotenoid (diets rich in e.g. tomatoes, watermelon, red grapefruit), with no vitamin A activity, may have benefit in preventing prostate cancer; doses of 6-30 mg.
  - Luteine, a carotenoid (diets rich in e.g. broccoli,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

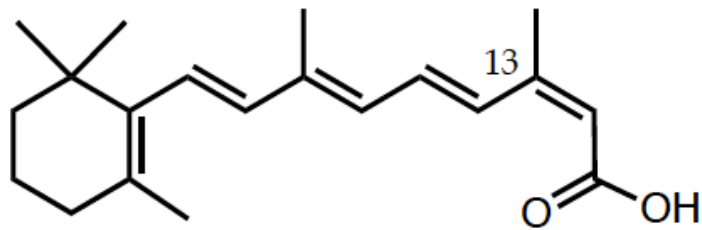
1st



all-*trans* Retinoic Acid  
TRETINOIN

**RAR ligand**

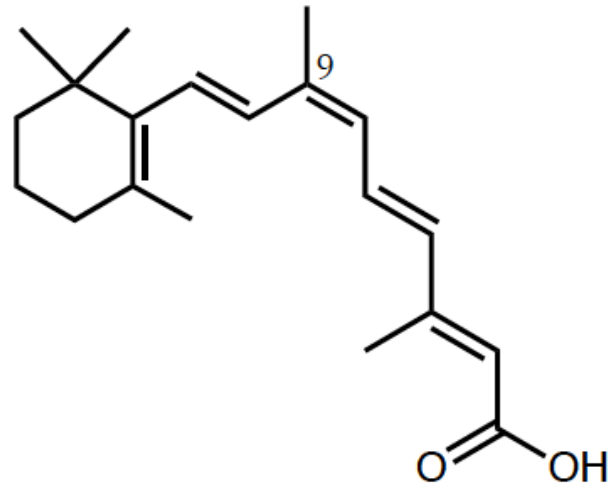
- PML - oral



13-*cis* Retinoic Acid  
ISOTRETINOIN

**RAR ligand  
(weak)**

- Severe nodular acne– oral
- IPLEDGE

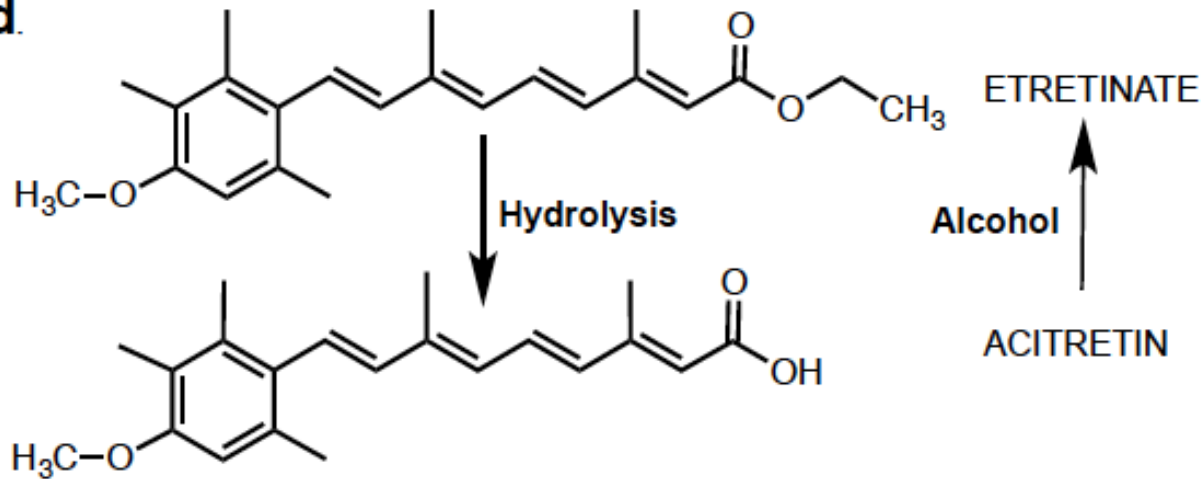


9-*cis* Retinoic Acid  
ALITRETINOIN

**RXR ligand**

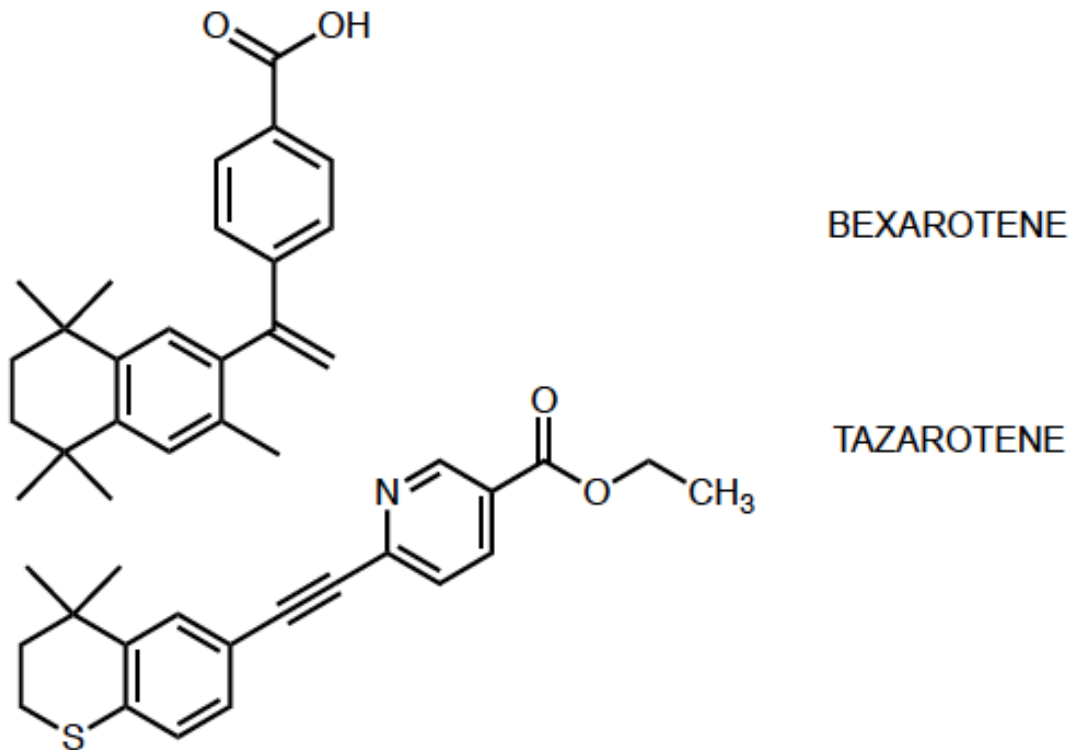
- Chronic eczema – oral
- Skin lesions in Kaposi's sarcoma – topical

**2nd.**



- Capsules removed from US market in 1998.
- Pro-drug, teratogenic with long half-life of 3-4 months.
- Teratogenic, but with shorter half-life of 2 days- so thought to be more 'manageable'.
- RAR ligand.

**3rd**



- Cutaneous manifestations of T-cell lymphoma.
- RXR ligand
- Psoriasis, acne.
- Prodrug for tazarotenic acid
- RAR ligand

## 9. Toxicity

- a. Teratogenic when taken in as retinyl palmitate/acetate in supplements??
  - This is controversial. Significant increase in the risk of birth defects (4-5 fold) has been observed at (preformed) 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. UL is only 10,000 IU/day
- 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.

## 1. Background

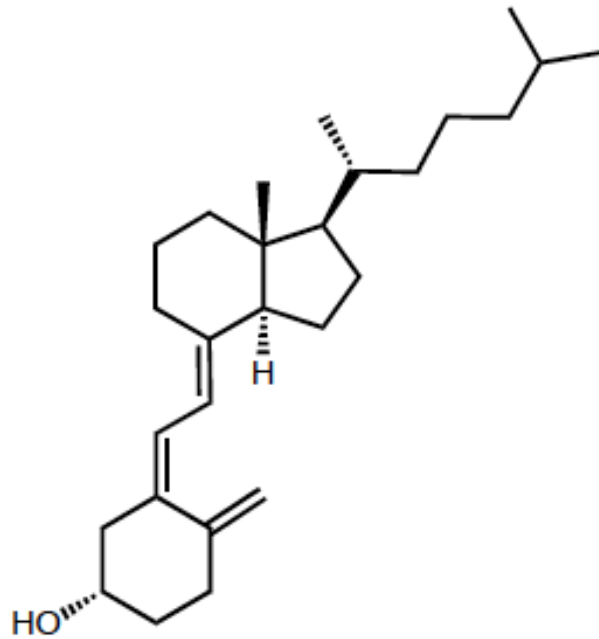
- Vitamin D has existed on earth for at least 500 million years, produced then in ocean-dwelling phytoplankton exposed to sunlight for photosynthesis. Might have functioned as the first 'sunscreen' or as a photochemical signaling molecule.
- Vitamin D is the anti-rachitic factor present in cod liver 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.



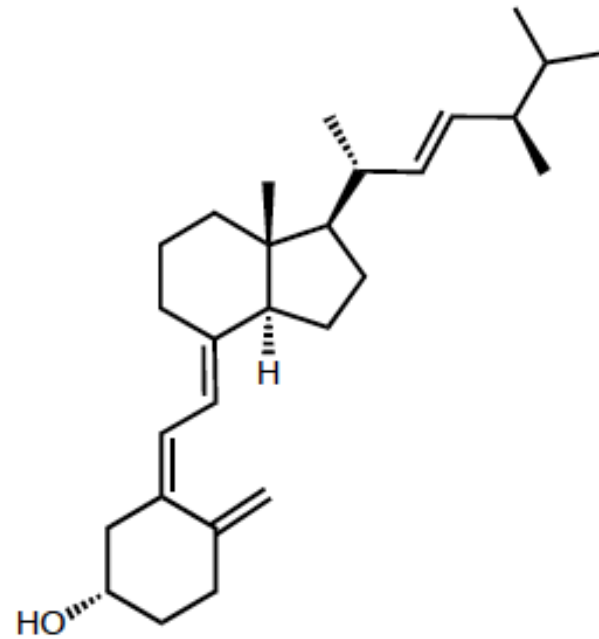
- In the 1920s, it was found that rickets could be treated with UV radiation. Importantly, it was found that irradiating one arm of a rachitic child cured rickets everywhere in the body, so this could not be just a 'local' phototherapy effect – a hormone?
- A hormone is a chemical substance produced 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

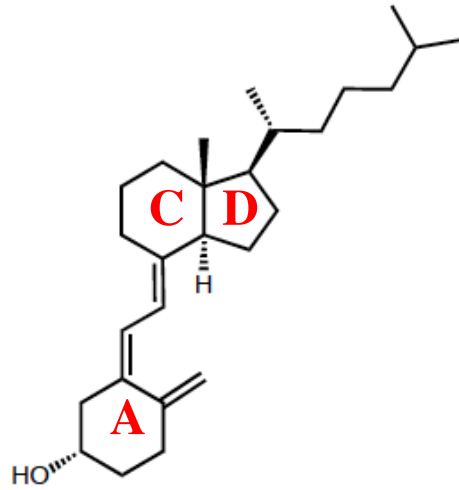


**D3 - Cholecalciferol**

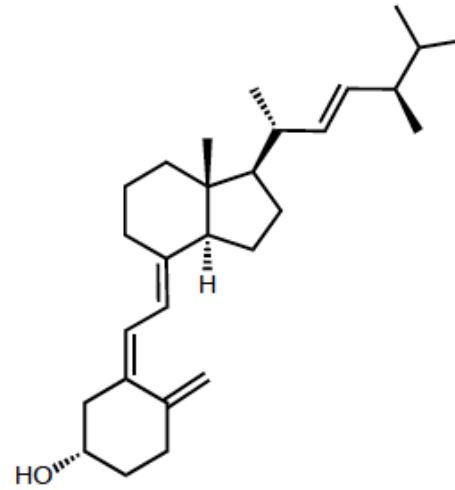


**D2 - Ergocalciferol**

- 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).



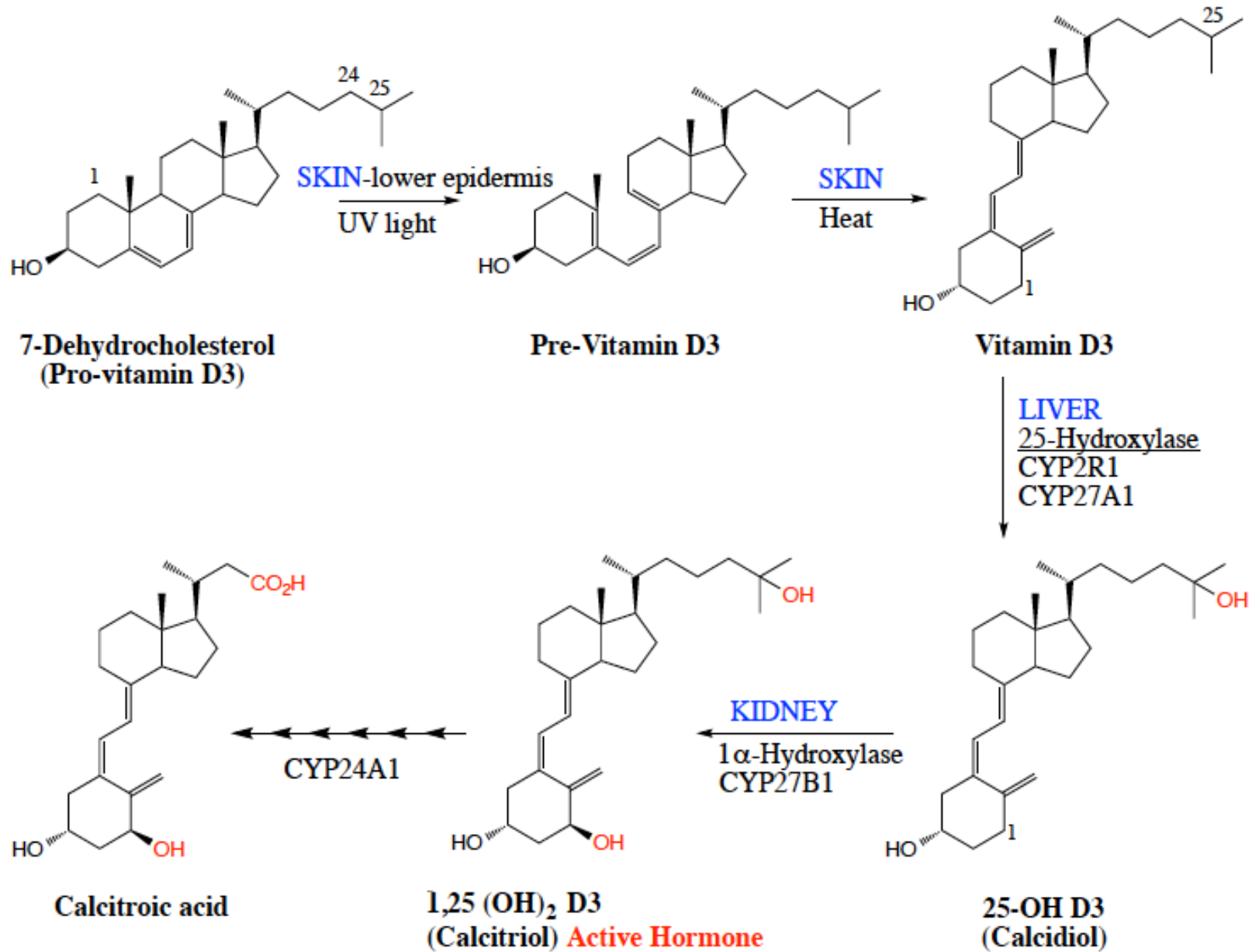
**D3 - Cholecalciferol**



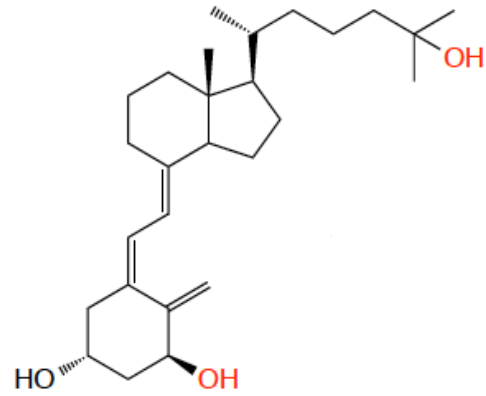
**D2 - Ergocalciferol**

- 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



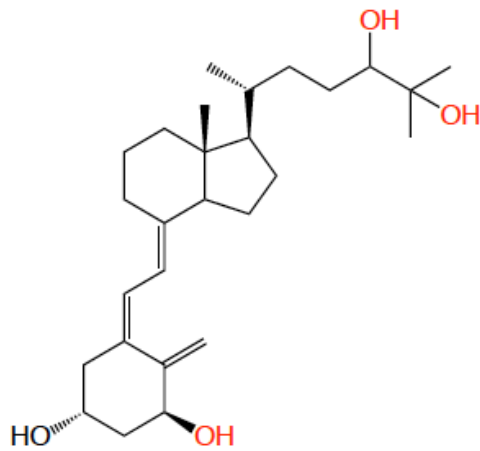
## Other inactivation pathways



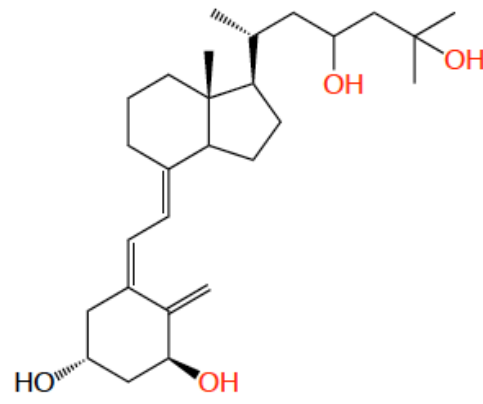
**1,25 (OH)<sub>2</sub> D3**  
**(Calcitriol) Active Hormone**

Inducible enzyme — CYP3A4  
CYP24A1

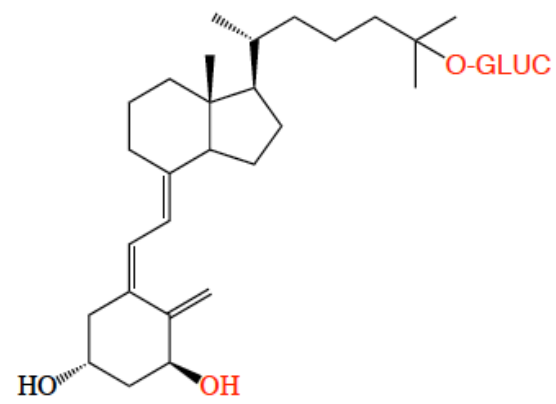
UGT1A4



**1,24,25 (OH)<sub>3</sub> D3**



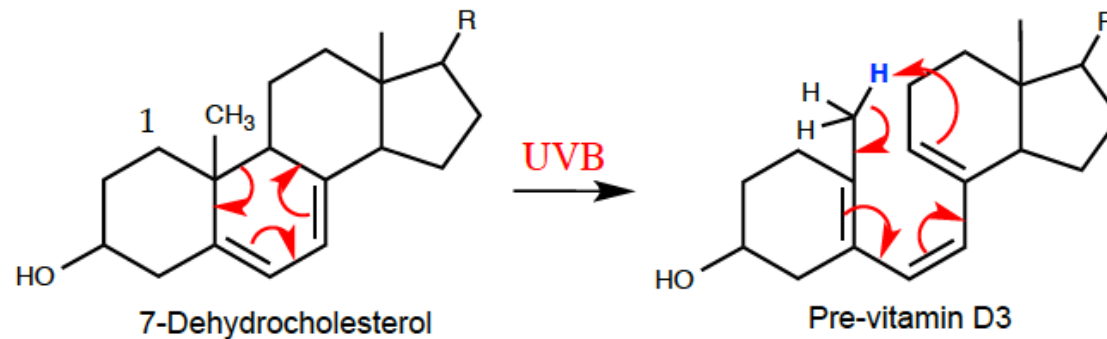
**1,23,25 (OH)<sub>3</sub> D3**



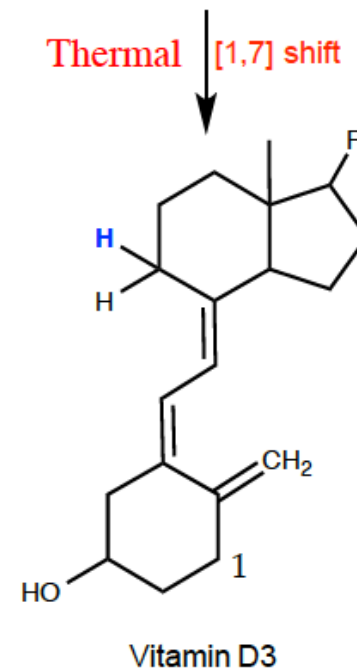
**1,25 (OH)<sub>2</sub> D3 Glucuronide**

## Photoactivation of 7-Dehydrocholesterol

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

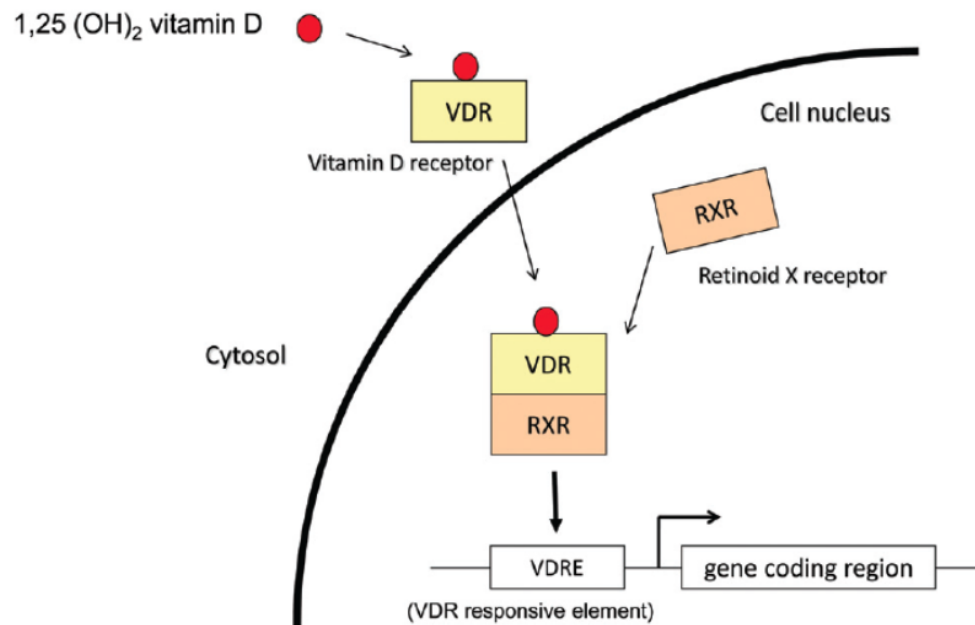


- UVB provides high energy photons that are absorbed into the B ring *via* the conjugated diene.



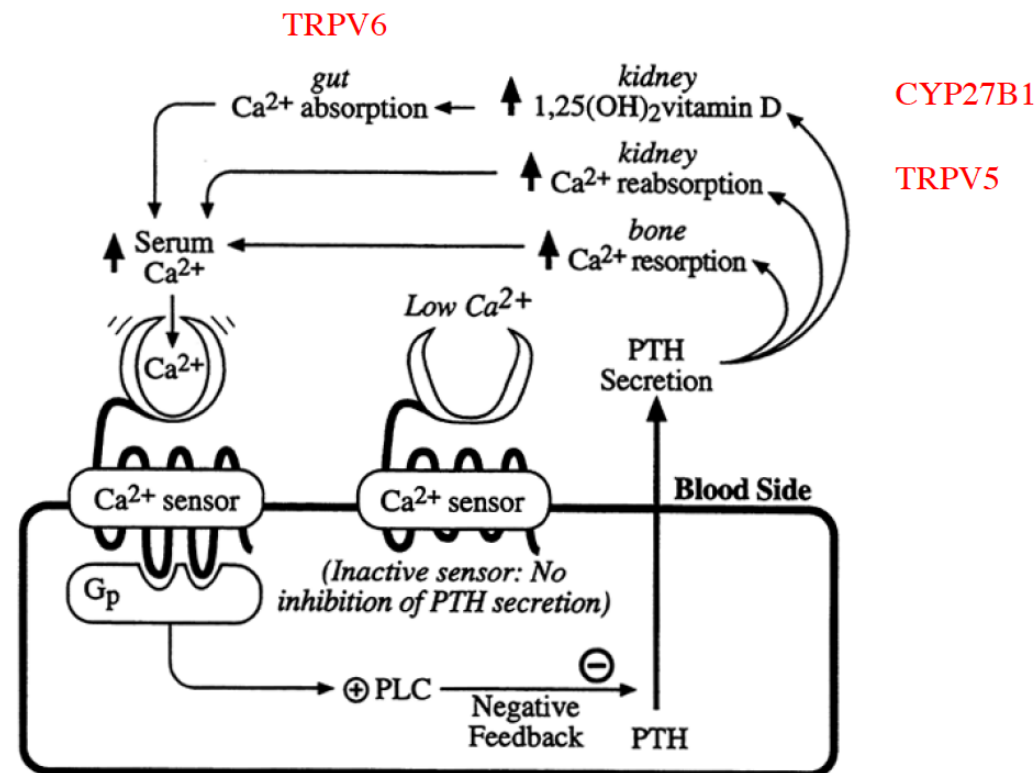
### 3. Function

- 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 Ca<sup>2+</sup> binding and transport proteins involved in Ca<sup>2+</sup> (and phosphate) absorption in the intestine and in Ca<sup>2+</sup> reabsorption from the kidney. TRPV6 and TRPV5, respectively.

- 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), 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.
- Osteomalacia – defect in bone-building process resulting in softened bones  
Osteoporosis – weakening of previously constructed bone

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.
    - ✓ 1,25 DHCC (*Rx* Rocaltrol and generic products) are used to provide this active metabolite directly.
    - ✓ Paricalcitol (*Rx* Zemplar) Structural modification of 1,25 DHCC used orally for hyperparathyroidism.
    - ✓ Cinacalcet (*Rx* Sensipar) **Not** a vitamin D analog. A positive allosteric modulator of the calcium sensor. Acts as a calcimimetic, turns off PTH release.

## 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.