UNIT 7 FAT-SOLUBLE VITAMINS: VITAMIN A, D, E AND K

Structure

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7.1 INTRODUCTION

Vitamins are *the organic substances that act as coenzyme and/or regulator of metabolic processes*. There are 13 known vitamins, most of which are present in foods while some are produced within the body. You would recall from your study in Nutritional Biochemistry Course that depending on the property of solubility, vitamins are divided into two groups, namely, *water-soluble* and *fat-soluble*. Water-soluble vitamins include vitamin B-complex, which is a group of *B Vitamins,* and *vitamin C* or *ascorbic acid* while the fat-soluble vitamins comprise of 4 vitamins- *A, D, E and K.* In this unit, we shall focus on the fat-soluble vitamins. The next unit shall deal with the water-soluble vitamins.

Fat-soluble vitamins A, D, E and K, are termed so because they are found in nature in close association with fatty foods such as butter, cream, vegetable oils, meat, poultry and fish and their products. Though these four vitamins have quite different properties, this unit discusses how they all share some commonalities such as mechanism of absorption from intestines, storage of the excess intake and development of deficiency with inadequate intakes, as well as, toxicity at intakes far in excess of the requirements. The unit also provides information on their requirements, status assessment and interaction with other nutrients.

Objectives

After studying this unit, you will be able to:

- describe the structure and functions of fat-soluble vitamins,
- identify their food sources, bioavailability and consequences of deficiency,
- recognize the recommended amount needed during various physiological stages, and
- appreciate their importance in relation to other nutrients.

7.2 FAT-SOLUBLE VITAMINS – AN OVERVIEW

What are fat soluble vitamins? As we already know, there are four fat-soluble vitamins – A, D, E and K. The presence of fat is required for the assimilation of these vitamins in the body. All of these, though quite different from each other in their structures, sources and physiological roles, are significant to us during different life stages. In this unit, therefore, we shall focus on understanding the following aspects for each of the vitamin:

- *Structure*: You would recall reading about the structures of these vitamins and their forms in the Nutritional Biochemistry Course (MFN-002) in Unit 3. Therefore, we are not going into the details in this unit and suggest you refer back to unit 2 in the Nutritional Biochemistry Course and refresh your understanding. For your convenience, however, we have given the structures of these vitamins here in the text. Look at Figure 7.1 which illustrates the fat-soluble vitamins. As you go through these structures, you would have noticed that all these fat-soluble vitamins have certain common features such as an aromatic ring structure with an aliphatic side chain, one or more double bonds either in the ring or in the side chain and a functional group such as an aldehyde (CHO : in vitamin A), ketone (C = O : in vitamin K), methyl (CH_3) or hydroxyl groups (OH : in vitamin D)

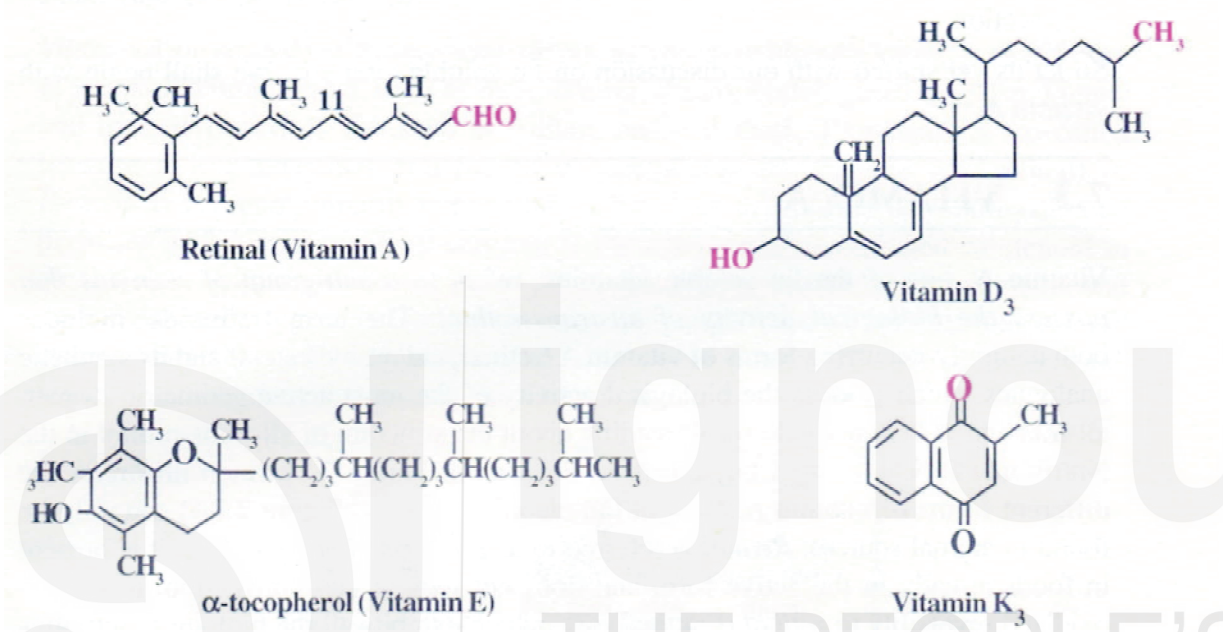


Figure 7.1: Fat-soluble vitamins

- *Food sources*: Under this sub-section, you will get to know the various food sources of these vitamins in our diet. As a student of dietetics, it is essential for you to know the foods that are rich in these vitamins.
- *Absorption, Storage and Elimination*: Reading through this section you would realize that all fat-soluble vitamins undergo similar metabolic fate. They are absorbed along with fats from the small intestine. Bile is essential for the effective absorption of fats and therefore fat-soluble vitamins. The salts of bile acids (taurine and glycine derivatives of cholic acid) are the digestion promoting constituents of bile. They are surface active agents (i.e. they lower the surface tension and emulsify fats) and also activate the enzyme *lipases*. They combine with fat-soluble vitamins to form molecular components which are then absorbed. Further, all fat-soluble vitamins are stored in concentrated amounts in the liver. The main pathway of excretion is through the bile into small intestine and consequently faecal excretion. A detailed discussion on absorption, storage of each vitamin is presented later, within this section.
- *Bioavailability*: The term bioavailability refers to *the overall efficiency of utilization, including physiological and biochemical processes involved in intestinal absorption, transport, metabolism and excretion of the nutrients*. In other words, it is the fraction of ingested vitamins absorbed and utilized for normal physiological functions or storage. Therefore, under this section we shall study the factors which affect the bioavailability of these vitamins.
- *Requirements and Recommended Dietary Allowances (RDA)*: What do we mean by requirements and RDA? The *requirement* level is the amount of nutrient needed to be absorbed to maintain adequate nutritional status in an individual.

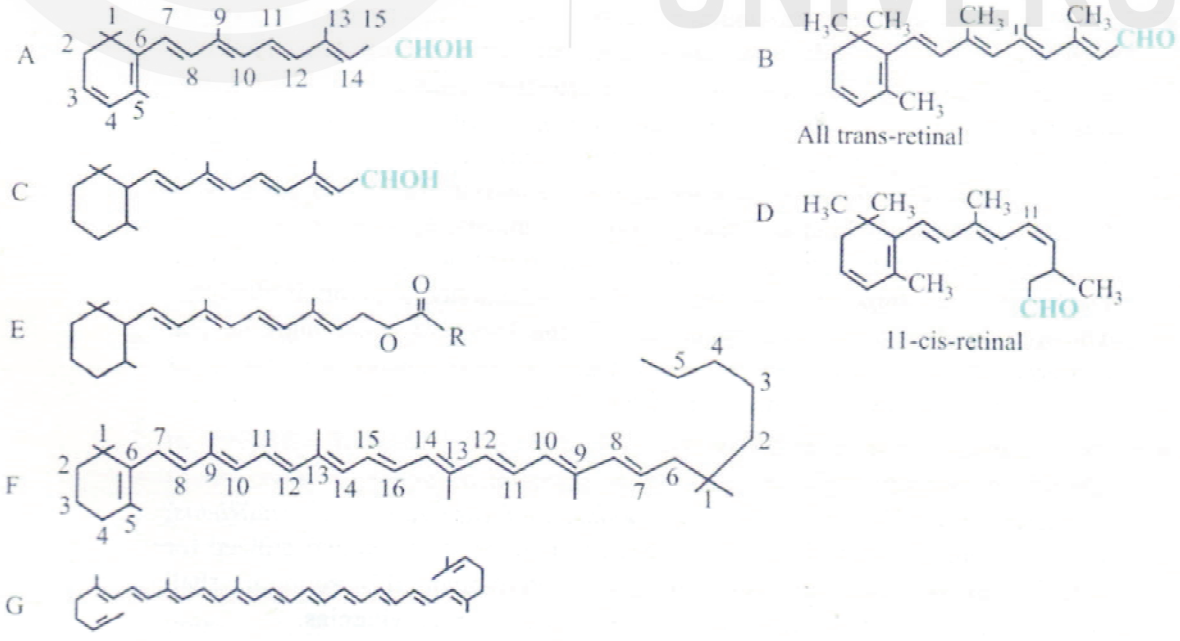
It differs with body size, age, rate of growth and special physiological situations such as pregnancy and lactation and some pathological conditions that accelerate usage, wastage or destruction as in certain acute or chronic diseases (diarrhoea/ constipation). *RDA*, on the other hand, is *the amount of a nutrient that will meet the needs of practically all individuals in a defined physiological category*. We shall get to know about the requirements and RDA of the fat-soluble vitamins in this section.

- *Hypo and Hypervitaminosis*: This section shall describe the adverse affects associated with deficient or excessive intake.
- *Criteria for assessing vitamin status*: The ability to accurately assess vitamin status requires criteria which are unique to the vitamin and will provide valid results fundamental to clinical and research settings. Such criteria are used to assess the nutriture of fat soluble vitamins, which are discussed here in this section.

So let us get started with our discussion on fat-soluble vitamins. We shall begin with vitamin A.

7.3 VITAMIN A

Vitamin A, one of the fat soluble vitamins, refers to *a sub-group of retinoids that possess the biological activity of all-trans-retinol*. The term 'retinoids' includes both naturally-occurring forms of vitamin A (retinol and retinyl esters) and its synthetic analogues which possess the biological activity of the most active geometric isomer, all-trans-retinol. You would recall reading about the structure of all-trans-retinol in the Nutritional Biochemistry Course in Unit 3. Look at Figure 7.2, which illustrates the different forms of vitamin A. Retinol (an alcohol) (refer to Figure 7.2A) can only be found in animal sources. *Retinol* is referred to as *pre-formed vitamin A*, as it is present in foods already in the active form and does not require any conversion. Retinol is oxidized reversibly to *retinal* (Figure 7.2B), which exhibits all the biological activities of retinol, or further oxidized to *retinoic acid* (Figure 7.2C), which is active in animal growth, but not in vision or reproduction. The form of vitamin A involved in vision is *11-cis-retinal* (refer to Figure 7.2D), whereas the primary storage forms are *retinyl esters* (refer to Figure 7.2E), the most common of which is *retinyl palmitate*.



A: all-trans retinol; B: all-trans retinal; C: all-trans retinoic acid;
D: 11-cis retinal; E: retinyl esters, mainly retinyl palmitate; F: all-trans β carotene; G: Lycopene

Figure 7.2: Different forms of vitamin A

The major source of vitamin A, as you may be aware, is the carotenoid pigments which are synthesized by plants. Several carotenoids possess vitamin A activity and include alpha, beta and gama carotene, lycopene (refer to Figure 7.2H) and cryptoxanthin to name a few. The primary and most efficient pro vitamin A or carotenoid is the *beta-carotene* (molecular weight 536.9) which has two molecules of retinal attached tail to tail. Unlike retinol, most carotenoids can quench singlet oxygen and act as antioxidants because of their long chain of conjugated double bonds, as can be seen in Figure 7.2G. Upon hydrolysis, each molecule of beta-carotene theoretically yields two molecules of vitamin A. The other carotenoid precursors are about half as active as β-carotene.

Next, let us study about the food sources of vitamin A.

Food Sources of Vitamin A

Vitamin A or retinol (*preformed vitamin A*), as you may already know, is found only in foods of animal origin, such as milk, cheese, cream, butter, ghee, egg, fish, kidney and liver, liver oils of fish such as halibut, cod and shark. *Provitamin A* (so called because it is a precursor and has to be chemically transformed into retinal) or *β-carotene* is found primarily in plant foods, which contain orange or yellow-coloured pigments called *carotenoids*. β-carotene is the most widely distributed carotenoid in plant foods. Palm fruit and red palm oil are the richest source of beta-carotene and dark green leafy vegetables, ripe fruits such as mango, papaya, apricots and yellow/orange vegetables like carrot, pumpkin and sweet potato are rich in β-carotene. Figure 7.3 illustrates the sources of vitamin A and beta-carotene.

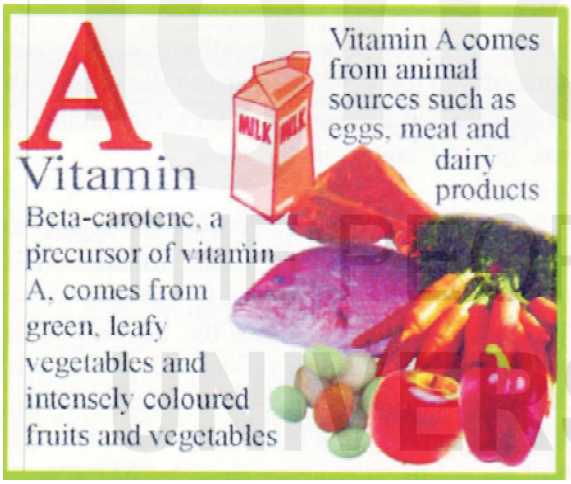


Figure 7.3: Food sources of vitamin A and beta-carotene

Now that we have looked at the sources, let us understand how vitamin A is absorbed and stored in our body.

Absorption, Storage and Excretion

From our discussion above, it is clear that the dietary supply of vitamin A consists of retinoids (retinol and retinyl esters in annual tissues) and carotenoids (β- carotene and other carotenoid pigments from plants). Let us see how these are absorbed.

Absorption

Vitamin A and carotenoids tend to aggregate with lipids into globules, which then pass into the small intestine. Dietary vitamin A (retinol) is absorbed as such in the intestines. Retinyl esters (mainly palmitate) are hydrolyzed by the combined action of bile salts and the *esterases* in the small intestine. The released carotenoids and retinol in the small intestine are solubilized into *miscelles* i.e., small aggregates of mixed lipids and bile salts suspended within the gastric bolus (material taken into the body by way of the digestive tract) solution. The micelles are absorbed into the intestinal mucosal cell. Approximately 70 to 90% of retinol from the diet is absorbed as long as the diet is

adequate in fat. Carotenoid absorption from the diet ranges from about 20% to 50%, but carotenoid absorption may be as low as 5%.

Transport and Utilization

The efficacy of the intestines to facilitate absorption and utilization of retinoids and carotenoids depends upon the cellular uptake of these compounds into the intestinal mucosal cell, bioavailability or passage of the molecules beyond the intestinal mucosa into the body with the potential for storage or use in tissues and bioconversion i.e., production of active retinoid from provitamin A carotenoids. Inside the intestinal cells, β-carotene is cleaved by a cytosolic enzyme *15–15' oxygenase* to form retinaldehyde, which is then reduced by the microsomal enzyme *retinal reductase* to retinal. Thus, the ultimate product formed in the intestines is retinol, which is then re-esterified with long chain fatty acids by intra cellular retinol-binding proteins (CRBP) and packed into chylomicron containing cholesterol esters, phospholipids, triacylglycerol etc. What happens to this chylomicra? Let's see this in the next paragraph how are these transported via circulation.

Transport

The chylomicra and retinal-binding protein play an important role in the transport of retinol. This chylomicra complex enters the lymphatic system via the thoracic duct and into systemic circulation. Chylomicrons deliver retinyl esters, some unesterified retinol and carotenoids to many extrahepatic tissues such as bone marrow, spleen, blood cells, lungs, kidney. Chylomicron remnants deliver retinyl ester and a portion of the carotenoids not taken by peripheral tissue to the liver.

For carotenoids reaching the liver, a small portion can be cleaved to form retinol, some may be incorporated into the very low-density lipoprotein (VLDLs) synthesized in the liver, and then be released as part of VLDLs for circulation to various tissues in the body and some may be stored in the liver.

As for the retinyl esters reaching the liver, hydrolysis of retinyl esters occurs. Within the cells, retinol binds with a cellular retinol-binding protein (CRBP). CRBP is thought to function both to help control concentration of free retinol within the cell cytoplasm and thus prevent its oxidation, and to direct the vitamin to specific enzymes of metabolism. The enzymatic metabolism of retinol includes esterification by enzymes such as *lecithin retinol acyl transferase* (*LRAT*) or *acyl CoA retinol acyl transferase* (*ARAT*), oxidation of retinol to retinal by NAD(P)H-dependent *retinol dehydrogenase*, and phosphorylation of retinol to retinyl phosphate by ATP for glycoprotein function.

Retinol not metabolized or transported from the liver may be stored in small cells called *stellate cells* (along with lipid droplets) following re-esterification.

Retinol mobilization from the liver and delivery to target tissue are dependent on the synthesis and secretion of retinol-binding protein (RBP). It would be interesting to note that RBP is a 183 amino acid residue, has a molecular weight of about 21,000 and is present in concentration at 3-4 mg/dl RBP, in turn, joins the binding site on a larger protein, transthyretin (TTR). Thus the hepatic parenchymal cell is involved in the uptake and storage of vitamin A in the liver and its release into circulation as the retinol-RBP-TTR complex. The retinol-RBP-TTR complex circulates in the plasma with a half-life of about 11 hours. Some tissues that take up retinol from the RBP-TTR complex include the adipose, skeletal, kidney, white blood cells and bone marrow.

Next, let us get to know about the storage of vitamin A in the body.

Storage

The primary organ for storage of vitamin A is the *liver*. Reserves are found in the stellate cells, as mentioned above. The average liver weighs 1.5 kg and when replete, contains 450 mg of vitamin A stores. Hepatic tissue concentrations of < 30 mcg / g is considered marginal. Total body stores of vitamin A range from 300-900 mg with 20% found in peripheral organs and tissues.

So far we have discussed about the absorption, transport, storage of vitamin A. It was mentioned in the text above that the bioavailability of vitamin A is a determinant of its absorption and hence excretion. Let us now understand the key aspects related to the bioavailability of vitamin A.

Bioavailability of Vitamin A

By now it is clear that vitamin A is supplied in two forms. One form is *retinol*, from animal foods such as liver, fatty fish, eggs, and milk, and from fortified foods. Retinol is considered *pre-formed vitamin A*. The other form is the *carotenoids* from plant foods (β-carotene, α-carotene and β-cryptoxanthin). These convert to vitamin A in the body and are called *provitamin A carotenoids*. Retinol and carotenoids have different vitamin A activity. You would realize that it takes greater amounts of carotenoids to meet the vitamin A requirement. The ICMR Expert Committee (2020) has revised the carotene conversion (CF) ratio to account for tissue concision and a general CF of 6:1 is recommended for β carotena and 12:1 for α-carotena and β-cyptoxanthene.

Having understood the concept of bioavailability of carotenoids, now let us look at the factors which influence the bioavailability of carotenoids.

Factors Affecting Bioavailability of Carotenoids

Factors affecting bioavailability of carotenoids can be classified under the following two headings:

1) *Factors influencing uptake from lumen to intestinal cells*
   - inhibition by intrinsic matrix,
   - inhibition by dietary fiber sources,
   - differential crowding by stereoisomeric forms,
   - inverse relationship between ingested amount and uptake,
   - intraluminal oxidative destruction,
   - enhancement by presence of fat and oil, and
   - enhancement by cooking and processing.
2) *Factors influencing the efficiency of bioconversion*
   - amount of provitamin A presented to the cell,
   - differential conversion by stereoisomeric form, and
   - vitamin A status of the host.

With a brief review of the factors, we end our discussion on bioavailability of vitamin A. Next, let us now move on to the functions of vitamin A.

Functions of Vitamin A

Vitamin A (retinol) is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system, growth and development, and maintenance of epithelial cellular integrity, immune function, and reproduction, as highlighted in Figure 7.4. While we discuss the major functions of vitamin A, it is important to note that primary vitamin A deficiency (VAD) may give rise to more than one secondary

effects which can often be recognized in the form of clinical signs and symptoms most important being ocular manifestations grouped under '*xerophthalmia*', (about which you may recall studying in the Public Nutrition Course (MFN-006) in Unit 3, sub-section 3.3.1). In addition to the specific signs and symptoms of xerophthalmia and the risk of irreversible blindness, nonspecific symptoms include increased morbidity and mortality, poor reproductive health, increased risk of anaemia, and contributions to depressed growth and development.

Let us then review the functions of vitamin starting with the most critical function of vitamin A i.e., its role for maintaining vision.

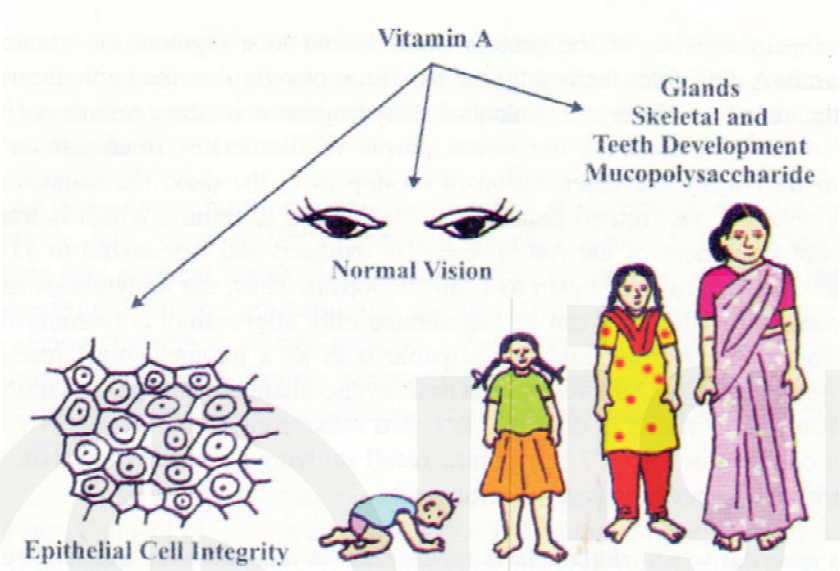


Figure 7.4: Functions of vitamin A

1) *Role in visual perception and function*: Vitamin A plays a critical role for maintaining normal vision. This function is of critical importance both from clinical relevance, as well as, public health point of view, as you may already be aware. Vitamin A deficiency is the leading cause of preventable severe visual impairment and blindness, and the most vulnerable are preschool children and pregnant women, particularly in our country. It is ironical that a small amount of less than 10 gm of fresh leaves can meet the days requirement of vitamin A of preschool children. Yet an estimated 2,50,000 to 5,00,000 VAD children world over become blind every year, and about half of them die within a year. Administration of large doses of vitamin A to children at-risk has been the most popular approach to control nutritional blindness.

But, how is vitamin A involved in the maintenance of vision? Our subsequent discussions will focus on this aspect.

The key component which interlinks vision with vitamin A is *rhodopsin* which is the photosensitive pigment of the eye and is also referred to as *visual purple*. In the visual system, as described above, carrier bound retinol is transported to ocular tissue and to the retina by intracellular binding and transport proteins. Rhodopsin, the visual pigment critical to dim-light vision, is formed in rod cells after conversion of all-trans-retinol to retinaldehyde, isomerization to the 11-cis-form, and binding to opsin. Alteration of rhodopsin through a cascade of photochemical reactions results in the ability to see objects in dim light, as illustrated in Figure 7.5. Let us understand these photochemical reactions in greater details.

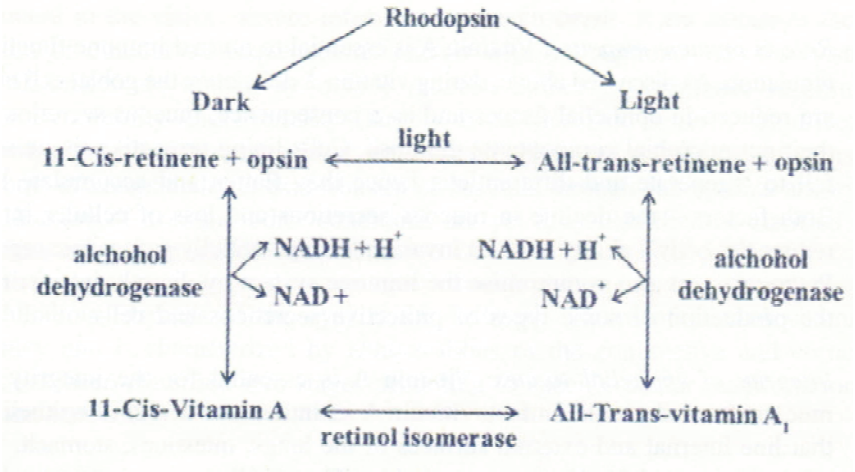


Figure 7.5: Rhodopsin cycle

Rhodopsin consists of the protein opsin bound to a pigment cis-isomer of retinal (vitamin A aldehyde) formed by the oxidation of retinol in the epithelium of the rods in the retina of the eye by alcohol dehydrogenase in the presence of NAD. The action of light bleaches the visual purple or dissociates rhodopsin to *opsin* and *retinene*. During the regeneration of rhodopsin in the dark, the sequence of events gets reversed, i.e., retinyl palmitate is hydrolyzed to retinol, which is transported to the outer segment of the rod. There it is oxidized and isomerized to 11-cis retinal, which then reacts with opsin to form rhodopsin. Thus, the re-synthesis of rhodopsin is isomer specific i.e. it can be regenerated only after retinol is rearranged to the 11-cis form. The rearrangement is possible both as a photochemical reaction and an oxygen-dependent dark reaction. These cyclic changes in which vitamin A plays a critical role in vision in dim light is called *Rhodopsin cycle* (vitamin A visual cycle), as is depicted in Figure 7.5. You may recall studying about the rhodopsin cycle in the Nutritional Biochemistry Course as well.

The speed at which rhodopsin is regenerated is related to the availability of retinol. Night blindness is usually an indicator of inadequate available retinol. Deficiency of vitamin A in the diet leads to impairment in the vision particularly at night or when dark. This is referred to as 'night blindness', when the individual cannot see in dim light. Difficulty in reading or driving a car in dim light, progresses to inability to see the objects in dim light. We shall read about the progressive stages of visual impairment with respect to vitamin A status in the human body later in this section.

The next function of great significance is the role of vitamin A in differentiation of cells.

2) *Role in growth and cellular differentiation*: The growth and differentiation of epithelial cells throughout the body are especially affected by vitamin A deficiency. In addition, goblet cell numbers are reduced in epithelial tissues and as a consequence, mucous secretions diminish. Cells lining protective tissue surfaces fail to regenerate and differentiate, hence they flatten and accumulate keratin. Classical symptoms of xerosis (drying or non-wetability) and desquamation of dead surface cells as seen in ocular tissue (i.e. xerophthalmia) are the external evidence of the changes also occurring to various degrees in internal epithelial tissues. Current understanding of the mechanism of vitamin A action within cells outside the visual cycle is that cellular functions are mediated through specific nuclear receptors. Binding with specific isomers of retinoic acid (i.e.all trans- and 9-cis-retinoic acid) activates these receptors. Activated receptors bind to DNA response elements located upstream of specific genes to regulate the level of expression of those genes. These retinoid-activated genes regulate the synthesis

of a large number of proteins vital to maintaining normal physiologic functions. There may, however, be other mechanisms of action that are as yet undiscovered.

3) *Role in immune response*: Vitamin A is essential to normal immune function and regulation. As discussed above, during vitamin A deficiency the goblet cell numbers are reduced in epithelial tissues and as a consequence, mucous secretions (with their antimicrobial components) diminish. Cells lining protective tissue surfaces fail to regenerate and differentiate; hence they flatten and accumulate keratin. Both factors – the decline in mucous secretions and loss of cellular integrity– reduce the body's ability to resist invasion from potentially pathogenic organisms. Pathogens can also compromise the immune system by directly interfering with the production of some types of protective secretions and cells.

4) *Integrity of epithelial tissues*: Vitamin A is essential for the integrity of the mucous-secreting cells. In fact, vitamin A maintains the health of epithelial cells that line internal and external surfaces of the lungs, intestines, stomach, vagina, urinary tract and bladder, eyes and skin. These cells act as important barriers to bacteria. Certain epithelial cells secrete mucous to keep the skin, eyes and other mucous membranes moist. In deficiency, the epithelial tissues are keratinized. The tissues affected are salivary glands, respiratory tract, eyes, skin and sex organs.

5) *Role as antioxidant*: Some carotenoids, in addition to serving as a source of vitamin A, have been shown to function as antioxidants. Studies show that *lycopene* (the pigment which gives tomatoes the red colour) is a scavenger of singlet-oxygen, offering powerful antioxidant activity. Antioxidants protect our cells against the effects of free radicals, which you may recall reading in the Nutritional Biochemistry Course, are potentially damaging compounds produced as by-products of metabolism, as well as, through exposure to toxins and pollutants (e.g. smoking).

   Free radicals, as you may be aware, can cause cell damage that may contribute to the development of cardiovascular disease and cancers. Thus, vitamin A and related nutrients may collectively be important in protecting against conditions related to oxidative stress, such as aging, air pollution, arthritis, cancer, cardiovascular disease, cataracts, diabetes mellitus and infection. However, this role has not been consistently demonstrated in humans.

6) *Bone and nerves*: The role of vitamin A in bone formation and the association of its deficiency with the degeneration of myelin sheath is currently being explored.

7) *Role in protein metabolism and growth*: Severe vitamin A deficiency results in abnormal RNA metabolism and protein synthesis and hence interferes with growth. Hence vitamin A is also called *growth vitamin*. Conversely, absorption and mobilization of vitamin A is impaired in protein malnutrition.

8) *Role in the synthesis of mucoproteins and macropolysaccharides*: Vitamin A is vital for the synthesis of mucoproteins and glycoproteins and incorporation of inorganic sulphate in mucopolysaccharides and their synthesis.

9) *Role in reproduction*: Deficiency of vitamin A leads to infertility in the male and failure of the female to conceive or resorption or abortion of the foetus, chiefly in animals.

After getting a thorough knowledge of the functions of vitamin A, let's get to know about the effects of its deficiency and excess.

Deficiency and Toxicity of Vitamin A

WHO defines VAD as *tissue concentrations of vitamin A low enough to have adverse health consequences even if there is no evidence of clinical xerophthalmia*. Vitamin

A deficiency (VAD), as you may already know, leads to impairment in the vision, severe infections and even death. It encompasses the full spectrum of clinical consequences associated with sub optimal vitamin A status. These disorders are known to include reduced immune competence resulting in increased morbidity and mortality (largely from increased severity of infectious diseases), night blindness, corneal ulcers, keratomalacia and related ocular signs and symptoms of xerophthalmia; exacerbation of anaemia through sub optimal absorption and utilization of iron and other conditions not yet fully identified or clarified (e.g. retardation of growth and development).

Xerophthalmia (dryness of the eye) is the hallmark feature of clinical vitamin A deficiency and is characterized by abnormalities of the conjunctiva and cornea of eye. It has been classified into stages according to specific ocular manifestations as described herewith:

One of the earliest manifestations of xeropthalmia is *night blindness* (*Stage XN*). Individuals suffering from night blindness cannot see in dim light or around dusk. Subsequently, the conjunctiva, which is the thin transparent membrane that covers the cornea and lines the inside of the eyelid, becomes discoloured (muddy coloured), dry and looses its brightness. This stage is known as *conjunctival xerosis* (*Stage XI A*). In addition to xerosis, dry, foamy, triangular spots may appear on the conjunctiva. These are known as the *Bitot's spot* (*Stage XI B*). Though conjunctival changes in xeropthalmia do not lead to blindness, they should be considered as warning signs. If neglected, the changes may progress affecting the cornea causing *corneal xerosis* (*Stage X 2*). In this condition, the cornea becomes dry and dull and appears like ground glass. This condition must be treated as an emergency. If it is not treated immediately with vitamin A, the individual can develop ulcers (sores) in the cornea (*Stage X 3A - corneal ulceration*) leading to the liquefication of cornea, a condition called *keratomalacia* (*Stage X 3 B*). Increasing softening of the corneas may lead to corneal infection, rupture (perforation) and degenerative tissue changes. This condition inevitably leads to irreversible blindness. Past involvement causing *corneal ulcers* (*Stage XS*) when healed leave white scars on the black portion of the eye which can interfere with normal vision. A globe destroyed by advanced keratomalacia is *xerophthalmic fundus* (*XF*). In addition, thickening of the hair follicles (follicular hyperkeratosis) is a cutaneous manifestation of vitamin A deficiency. You may have already studied about this classification in the Public Nutrition Course in Unit 3.

Conditions and populations associated with increased need for vitamin A includes *young children particularly the pre-schoolers, pregnant and lactating mothers* as already highlighted earlier, as well as, clinical conditions such as malabsorptive disorders (steatorrhoea), pancreatic, liver or gallbladder diseases. Patients with chronic nephritis, acute protein deficiency, intestinal parasites, or acute infections may also become vitamin A deficient.

So far we have focused on the deficiency symptoms. What would be the consequences if we were to consume vitamin A more than the body required? Read the next sub-section and find out.

Toxicity

Because vitamin A is fat-soluble and can be stored, primarily in the liver, routine consumption of large amounts of vitamin A over a period of time can result in toxic symptoms, including liver damage, bone abnormalities and joint pain, alopecia, headaches, vomiting and skin desquamation. In fact, symptoms that occur due to intakes in excess of those recommended over a prolonged period are referred to as symptoms of *hypervitaminosis*. Hypervitaminosis A appears to be due to abnormal transport and distribution of vitamin A and retinoids caused by overloading of the plasma transport mechanisms. Very high single doses can cause transient acute toxic symptoms that may include bulging fontanelles in infants, headaches in older children and adults, and vomiting, diarrhoea, loss of appetite and irritability in all age groups. Rarely does toxicity occur from ingestion of food sources of preformed vitamin A.

When this occurs, it usually results from very frequent consumption of liver products. Toxicity from food sources of provitamin A, chiefly carotenoids, is not reported, except for the cosmetic yellowing of skin.

Most children aged 1-6 years tolerate single oral doses of 60,000 µg (2,00,000 IU) vitamin A in oil at intervals of 4-6 months without adverse symptoms. Occasionally diarrhoea or vomiting is reported but these symptoms are transient with no lasting sequelae. Older children seldom experience toxic symptoms unless they habitually ingest vitamin A in excess of 7,500 µg (25,000 IU) for prolonged periods of time. When women take vitamin A at daily levels of more than 7,500 µg (25,000 IU) during the early stages of gestation, foetal anomalies and poor reproductive outcomes are reported. Women who are pregnant or might become pregnant should avoid taking excessive amounts of vitamin A. A careful review of the latest available information by a WHO Expert Group recommended that daily intakes in excess of 3,000 µg (10,000 IU) or weekly intakes in excess of 7,500 µg (25,000 IU) should not be taken at any period during gestation.

From our discussions above, it is clear that both deficient and excess intakes of vitamin A can be harmful. So then what are the requirements of vitamin A by our body and what should be the most optimum intake to maintain good health? Let us read and find out.

Requirement and Recommended Dietary Allowance (RDA) for Vitamin A

Recommendations for adequate vitamin A intake are based on the amounts needed to correct night blindness among vitamin A deficient subjects and to raise plasma levels in vitamin A deficient individuals to a normal level. Vitamin A requirement (EAR and RDA) by age group is given in Table 7.1. Considering the recent studies on vitamin A status carried out in India, an upward revision of retinol to 900 µg is recommended during pregnancy. To ensure adequacy at least in vulnerable groups like pregnant and lactating women, the committee has recommended that a minimum of 50% RE be drawn from animal sources.

Table 7.1: Vitamin A requirements (EAR & RDA) by Age Group

Physiological group	Body weight (kg)	RDA 2020	
		EAR (µg /day)	RDA (µg /day)
Adult-Male	65	460	1000
Adult-Female	55	390	840
Pregnant	55.0+GWG	406	900
Lactating	55.0	720	950
Infants (0-6 mo)	5.8	-	350 (AI)
Infants (6-12 mo)	8.5	170 (AI)	350 (AI)
Children (1-3y)	11.7	180	390
Children (4-6y)	18.3	240	510
Children (7-9y)	25.3	290	630
Boys (10-12y)	34.9	360	770
Girls (10-12y)	36.4	370	790
Boys (13-15y)	50.5	430	930
Girls (13-15y)	49.6	420	890
Boys (16-18y)	64.4	480	1000
Girls (16-18y)	55.7	400	860

Source: RDA and EAR- Nutrient Requirements for Indians, ICMR (2020), NIN
RAE: Retinol Equivalents (µg/d); GWG: Gestational weight gain
AI : Adequate Intake

Criteria for Assessment of Vitamin A Status

Various parameters/criteria can be used for the assessment of vitamin A status. Some of these have been described herewith:

1) *Clinical Assessment*

Clinical features of deficiency occur as *ocular* and *extraocular lesions*. *Ocular lesions* affect the posterior segment of the eye initially with impairment of dark adaptation and night blindness (twilight blindness). Xerosis of the conjunctiva is the first sign seen on clinical examination. This leads to Bitot's spots. Keratomalacia is the last stage. These ocular lesions have already been described in section 7.3 above.

Extra-ocular lesions include dry scaly skin (follicular hyperkeratosis), toad skin or phrynoderma. There is increased susceptibility to infections. The WHO classification for assessment of vitamin A status, as described above in section 7.3, is summarized in Table 7.2.

Table 7.2: WHO classification for assessment of vitamin A status

Classification	Primary Signs
XI A	Conjunctival Xerosis
XI B	Bitot's Spots
X 2	Corneal Xerosis
X 3A	Corneal Ulceration
X 3B	Keratomalacia
	Secondary signs
X N	Night blindness
X F	Fundal changes
X S	Corneal scarring

2) *Conjunctival Impression Cytology (CIC)*

Conjunctival impression cytology (CIC) is a simple, rapid and inexpensive method which is suitable for a field survey. By touching with a filter paper the lower temporal portion of the conjunctiva for about 3-5 seconds, as illustrated in Figure 7.6, the desquamated layers of cells are transferred to the filter paper. This strip is then stained and examined. CIC is a useful test for the assessment of subclinical VAD. It detects the progressive loss of goblet cells in the conjunctiva and the appearance of enlarged, partially keratinized epithelial cells.



Figure 7.6: Conjunctival impression cytology

CIC provides an early measure of the histological changes in the eye i.e. changes in the conjunctional epithelium with eventual keratinization. These changes are used to differentiate between normal children and those with mild xerophthalmia. Diagnosis is based on the absence or rare appearance of goblet cells or mucin spots in sufficient quantity. The CIC technique is modified by another method in which cells are

immediately transferred to a glass slide. This method is called *conjunctival impression cytology with transfer*.

3) *Dietary Assessment Criteria*

Several methods can be adopted for the dietary assessment of vitamin A such as food frequency, weekly or 3 day food weighment record, and 3 day or 1 week food recall method.

4) *Serum Vitamin A Content*

Assessment of serum vitamin A content is the most reliable criterion for assessing vitamin A status. Serum levels indicative of various degrees of deficiency are as follows:

Status	Serum vitamin A levels (mcg/dl)
Normal	≥ 25
Deficiency	< 12
Sub clinical	12 - 25

Circulating vitamin A concentrations become elevated (>200 mcg / dl) owing to vitamin A overload.

5) *Liver Biopsy Assays*

These are used to measure the total vitamin A stores, as well as, the response in levels of vitamin A relative to different dosages of the vitamin. This method has an unacceptably high level of invasiveness and risk.

6) *Dark Adaptation*

In the early stages of VAD, the individual cannot see objects in dim light. This phenomenon is used as a criterion for assessment in the dark adaptation test. The subject is either kept in a dark room for some time and asked to identify an object which is dimly illuminated, the intensity of light being increased till the subject is able to see the object (or) he is exposed to bright light for some time, by which the visual purple is bleached. The time required for the rhodopsin to be regenerated is measured by the ability to see a dimly illuminated object. Longer time taken to identify the objects is indicative of variable stages of vitamin A deficiency.

With this, we end our study on assessment criteria of vitamin A status. Finally, we will focus on interaction of vitamin A with other nutrients. Let us get to know on this topic.

7) *Interaction with Other Nutrients*

Of the various nutrients, the interaction of vitamin E, proteins, zinc and iron with vitamin A is of significance. How? Let's proceed with our discussion and find out.

- *Vitamin E*: Vitamin E is required for the cleavage of β-carotene into retinal and to protect the oxidation of these compounds.

- *Proteins*: The protein status of an individual influences vitamin A status and transport because an inadequate protein intake depresses the activity of the enzyme that cleaves β-carotene. Also, vitamin A metabolism is closely related to protein status as vitamin A transport is dependent on several vitamin A-binding proteins synthesized in our body as discussed earlier.

- *Zinc*: Its deficiency interferes with vitamin A metabolism. It leads to a reduction in the synthesis of plasma proteins, particularly, RBP, made in the liver. Thus,

plasma retinol concentrations decrease and liver retinol concentrations increase. Also, zinc deficiency decreases hepatic mobilization of retinol from its storage form as retinyl esters.

- *Iron*: Iron status of an individual correlates with vitamin A. The deficiency of vitamin A has been found to be associated with microcytic anaemia. While the exact mechanisms underlying the impact of vitamin A on iron and anaemia are unknown, several hypothesis exist to explain this phenomenon. One prevalent hypothesis is that vitamin A increases levels of serum iron, which allows haematopoesis to thrive, increasing haemoglobin and erythrocyte production. In vitamin A deficiency, iron would not be available for erythropoesis, and anaemia would result.

  Another hypothesis, proposed by *Thurnham*, involves the immune function of vitamin A. *Thurnham* suggests that the anti-infectious activity of vitamin A could have an impact on the reversal of anaemia.

We end our study on vitamin A, the fat-soluble vitamin here. Let us now recapitulate what we have learnt so far.

Check Your Progress Exercise 1

1) Fill in the blanks:

   i) One of the major role of vitamin A is ..... .

   ii) The enzyme ....., ... oxidizes retinol to retinene in the retinal rods.

   iii) ..... is also called growth vitamin.

   iv) Vitamin A stimulates the formation of the protein ..... which facilitates calcium deposition in the bone.

   v) The requirement for vitamin A during infancy is ..... mcg and for a preschooler is ..... mcg per day.

   vi) Serum retinol content indicative of sub clinical VAD ranges from ..... to ..... mcg/dl.

2) What are retinoids? How do β-carotene differ from retinoids.

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3) Discuss the role of vitamin A in visual perception.

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4) List any five factors that affect bioavailability of carotenoids.

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5) What level of intake elicits acute vitamin A toxicity? What are the signs and symptoms of acute hypervitaminosis A?

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6) Enumerate the methods for assessing vitamin A status.

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Next, let us study about vitamin D, the other fat-soluble vitamin.

7.4 VITAMIN D

Vitamin D is a generic term and indicates a molecule of the general structure with rings (A, B, C, D), as you may have noticed in Figure 7.1 B. The ring structure is derived from the cyclopentanoper hydrophenenthrene ring structure for steroids, as you may recall studying in the Nutritional Biochemistry Course (MFN-002). *Ergocalsiferol (D_2), cholecalciferol* (D_3) are the forms of vitamin D.

Vitamin D, can either be made in the skin from a cholesterol-like precursor (7-dehydrocholesterol) by exposure to sunlight or can be provided pre-formed in the diet. The version made in the skin is referred to as *vitamin D_3* whereas the dietary form can be either vitamin D_3 or a closely-related molecule of plant origin known as *vitamin D_2*. Figure 7.7 shows the formation of Vitamin D_3 from its precursor 7-dehydrocholesterol. The synthesis of vitamin D_3 from its provitamin, 7-dehydrocholesterol, occurs by UV irradiation and proceeds from the provitamin to the previtamin and finally to the vitamin. The first main step of the reaction is the photochemical conversion of 7-dehydrocholesterol to previtamin D_3. This reaction yields upto 85% of previtamin D_3. The next and final step is accomplished by the thermal conversion of previtamin D_3 to vitamin D_3.

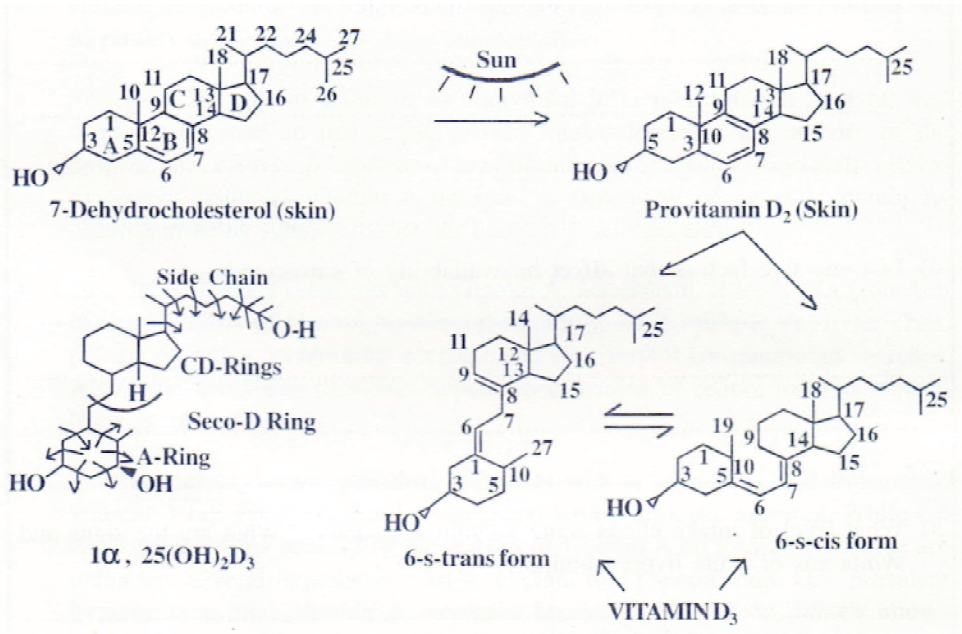


Figure 7.7: Pathway for the production of vitamin D_3

Next, let us review the food sources of vitamin D.

Sources of Vitamin D

Vitamin D, also called the sunshine vitamin is easily manufactured in the skin from 7-dehydro cholesterol on exposure to sunlight, as already mentioned above and also highlighted in Figure 7.8. Small amounts are present in dairy products such as milk, cheese, butter, margarine and cream, egg yolk, liver, oysters and certain varieties of fish. So we have seen that it is not just through diet, sunlight can also help us to manufacture vitamin D. We just read that the form of vitamin D present in food is different from that required by our body. So, how our body utilizes it? Let us read and find out next.

Absorption, Storage and Elimination

As we have already mentioned earlier, all fat-soluble vitamins share a common metabolic fate. Vitamin D is absorbed along with fats from the duodenum and jejunum. Bile too is essential for the effective absorption of fats and therefore of vitamin D.

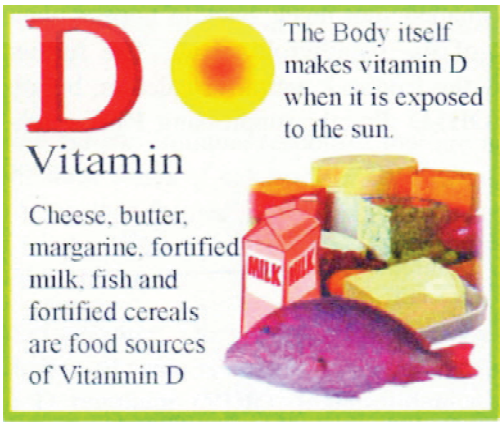


Figure 7.8: Vitamin D sources

Conditions unfavourable to fat absorption such as lack of bile, disorders such as sprue and celiac disease results in poor absorption of vitamin D. Once absorption is complete, vitamin D enters the blood as a part of the chylomicrons. Vitamin D formed in the skin by the direct irradiation of the provitamin present in the skin is directly absorbed into the blood stream. The vitamin is stored in concentrated quantities in the liver and to a lesser extent in the skin, spleen, lungs, brain and kidney. The main pathway of excretion of vitamin D is through the bile into the small intestine and consequent faecal

excretion. Only less than four per cent of the intake of the vitamin is excreted by the urinary pathway.

Next, let us now move on to the functions of vitamin D in our body.

Functions of Vitamin D

Vitamin D is required to maintain normal blood levels of calcium and phosphate, which are in turn needed for the normal mineralization of bone, muscle contraction, nerve conduction and general cellular functions in all cells of the body.

Vitamin D also modulates the transcription of cell cycle proteins, which decrease cell proliferation and increase cell differentiation of a number of specialized cells of the body (e.g. osteoclastic precursors, enterocytes, keratinocytes). This property may explain the actions of vitamin D in bone resorption, intestinal calcium transport and skin. Vitamin D also possesses immunomodulatory properties that may alter responses to infections *in vivo*. We shall review these functions in greater details in this section.

1) *Mobilization of bone calcium and phosphorous*: It is now firmly established that vitamin D_3 is metabolized first in the liver to 25-hydroxyvitamin D (25-OH-D) (calcidiol) and subsequently in the kidneys to *1,25 dihydroxycholecalciferol* or *1,25-$(OH)_2D$* (calcitriol) to produce a biologically active hormone. The functions of vitamin D are mediated by this vital vitamin D hormone by a homeostatic mechanism which involves the hormone acting on the intestines, kidney and bone to increase serum calcium and phosphorus levels. 1,25-$(OH)_2D$ stimulates intestinal absorption of calcium and phosphate and mobilizes calcium and phosphate by stimulating bone resorption. These functions serve the common purpose of restoring blood levels of calcium and phosphate to normal when concentrations of the two ions are low. This helps to achieve a normal blood calcium concentration and maintenance of *calcium homeostasis*.

In calcium homeostasis, 1,25-$(OH)_2D$ works in conjunction with parathyroid hormone (PTH) to produce its beneficial effects on the plasma levels of ionized calcium and phosphate. The physiologic loop (Refer to Figure 7.9) starts with the calcium receptor of the parathyroid gland. When the level of ionized calcium in plasma falls, PTH is secreted by the parathyroid gland and stimulates the tightly regulated renal enzyme *25-OH-D-1-α-hydroxylase* to make more 1,25-$(OH)_2D$ from the large circulating pool of 25-OH-D. The resulting increase in 1,25-$(OH)_2D$ (with the rise in PTH) causes an increase in calcium transport within the intestine, bone and kidney. All these events raise plasma calcium levels back to normal, which in turn is sensed by the calcium receptor of the parathyroid gland. The further secretion of PTH is turned off not only by the feedback action of calcium, but also by a short feedback loop involving 1,25-$(OH)_2D$ directly suppressing PTH synthesis in the parathyroid gland.

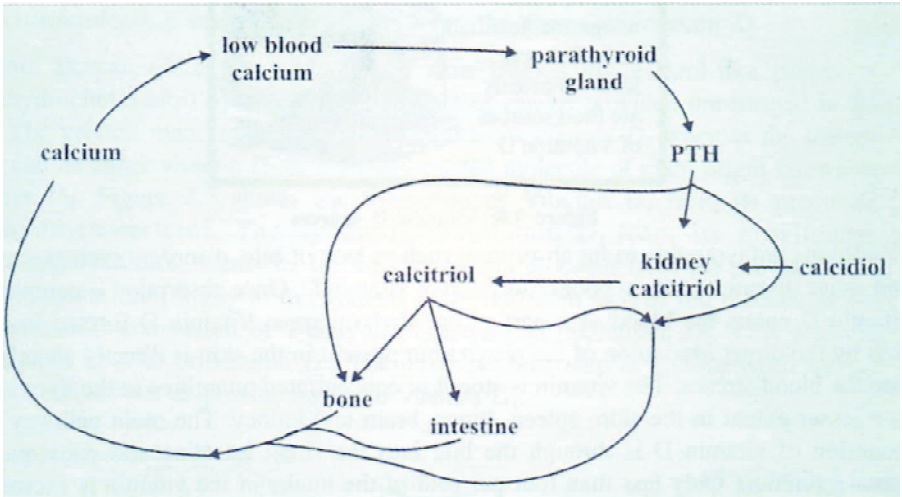


Figure 7.9: Calcium homeostasis

Source: Vitamin and mineral requirements in human nutrition. FAO/WHO 2004.

You may recall studying about how vitamin D helps in calcium homeostasis also in the Unit 10, section 10.3, sub-section 10.3.2 in the Nutritional Biochemistry Course.

2) *Mineralization and formation of new bone*: Vitamin D plays a role in the synthesis of a prominent non collagenous protein, *osteocalcin*, a vitamin K-dependent protein found in the bone matrix and dentine – which is associated with new bone formation.

3) *Bone growth and development–calcification of osteoid tissue*: Vitamin D participates in metabolic processes associated with bone growth and development. It is involved in calcification of *osteoid* tissues. Osteoid is a protein mixture which is secreted by osteoblasts. When it mineralizes, it becomes bone.

4) *Modulation of the transcription of cell cycle proteins*: The 1,25-$(OH)_2$D compound (calcitriol), is present in the blood complexed to the vitamin D-binding protein, a specific α-globulin. Calcitriol is believed to act on target cells in a similar way to a steroid hormone. Free hormone crosses the plasma membrane and interacts with a specific nuclear receptor known as the vitamin D receptor, a DNA-binding, zinc-finger protein with a relative molecular mass of 55,000. This ligand-receptor complex binds to a specific vitamin D-responsive element and, with associated transcription factors (e.g. retinoid X receptor), enhances transcription of mRNAs which code for calcium-transporting proteins, bone matrix proteins, or cell cycle-regulating proteins.

5) *Formation of enzymes*: Vitamin D is essential for the formation of two enzymes-a*lkaline phosphatase* in the intestinal lining (involved in calcium transport) and *adenosine triphosphatase*, (for collagen formation in bone matrix).

6) *Regulation of amino acid levels in the blood*: Vitamin D helps to prevent loss of amino acids through the kidney and thus regulate the amino acid level and also regulate the level of citric acid in tissues and bones.

7) *Participation in muscle formation and metabolism*: Vitamin D takes part in muscle function and metabolism.

8) *Inhibition of cancer cell proliferation and growth*: Vitamin D diminishes proliferation of abnormal intestinal, lymphatic, mammary and skeletal cells and provides a potential for the treatment of skin diseases such as *psoriasis* (a disorder in which there is proliferation of the keratinocytes and a failure to differentiate rapidly).

9) *Role in the immune system*: Immune responses that are mediated by T-cells can be inhibited by the large doses of calcitriol i.e. 1,25 dihydroxycholecalciferol. It is a natural steroid hormone formed in the healthy body as the biologically active form of vitamin D. A deficiency of vitamin D also interferes with the T-cell mediated immunity.

10) *Regulation of blood pressure*: The renin-angiotensin system regulates the blood pressure. The synthesis of renin is decreased by calcitriol through its interaction with the vitamin D regulator (VDR). Inappropriate activation of the renin-angiotensin system is thought to play a role in some forms of human hypertension and adequate vitamin D levels may be important for decreasing the risk of high blood pressure.

So, now we have a good idea about the role of vitamin D in our body. Next, let us study the factors which affect the bioavailability of vitamin D.

Bioavailability of Vitamin D

The nutritional availability of vitamin D is less significant because it can be endogenously produced and retained for long periods by the tissues. Factors that affect availability are the duration of exposure to sunlight, seasonal variation, skin pigmentation, cultural practices like purdah system associated with clothing that covers the entire body and face.

You might have seen small children with bow legs, who are unable to walk normally and have enlarged bone joints. These, along with many other symptoms, are associated with vitamin D deficiency. Let us have a look at the deficiency symptoms in further detail. Also, the toxicity aspect is discussed next.

Deficiency and Toxicity of Vitamin D

Infants constitute a population at-risk for vitamin D deficiency because of relatively large vitamin D needs brought about by their high rate of skeletal growth. Breast-fed infants are particularly at-risk because of the low concentrations of vitamin D in human milk. This problem is further compounded in some infants fed human milk by a restriction in exposure to ultraviolet (UV) light for seasonal, latitudinal, cultural or social reasons.

Dietary absence of vitamin D or lack of UV (sunlight) exposure causes the bone disease called *rickets* (refer to Figure 7.10) in infants/children and *osteomalacia* in adults. Let us first study the characteristic features of rickets in children.

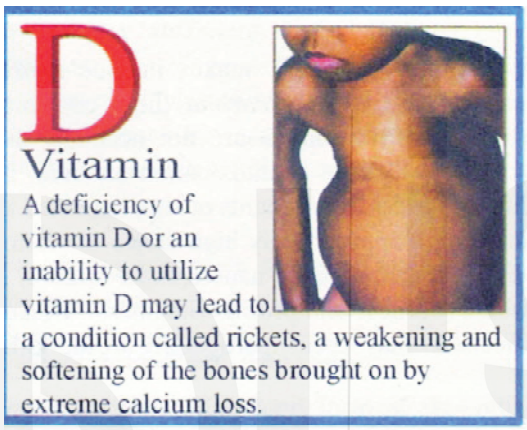


Figure 7.10: Rickets

Rickets: The following characteristics are seen in fully developed cases of rickets:

1) In case of young infants, delayed closure of the fontanelles i.e. a soft membranous gap between the cranial bones, softening and reduced mineralization of the skull (craniotabes).
2) While in older infants, sitting and crawling are delayed and there is bossing of skull. Also there are soft, fragile bones, bow legs, enlargement of the costochondral junction (a cartilage that attaches the front of the ribs to the breastbone) with rows of knobs or beads forming the Rachitic Rosary, pigeon chest and spinal curvature.
3) Enlargement of wrist, knee (knock-knees) and ankle joints.
4) Poorly developed muscles, lack of muscle tone, pot belly being the result of weakness of abdominal muscles, weakness with delayed walking.
5) Restlessness and nervous irritability.
6) High serum alkaline phosphatase, low inorganic blood phosphorus, normal or low serum calcium.
7) Tetany characterized by low serum calcium, muscle twitching, cramps and convulsions.
8) Delayed dentition and malformation of the teeth, permanent teeth more subject to decay.

Let us now discuss the symptoms of 'adult rickets' i.e. *osteomalacia*.

Osteomalacia: It occurs when there is a lack of vitamin D and calcium, in women who have had many pregnancies, who subsist on a meagre cereal diet with little exposure to sunshine. In osteomalacia, the following changes are seen:

1) Softening or demineralization of the bones leading to deformities of legs, spine, thorax and pelvis. As the bones soften, weight may cause bowing of the long bones, vertical shortening of the vertebrae and flattening of pelvic bone.
2) Rheumatic pain in bones of the legs and back.
3) General weakness with difficulty in walking.
4) Spontaneous multiple fractures.
5) Normal parturition difficult since sacrum convexity is increased, ribs of the iliac bone are flattened and the inlet becomes asymmetrical and narrowed.

Now that you have well understood the consequences of vitamin D deficiency, let us have a look at the signs and symptoms of its excessive amounts in our diet.

Toxicity

The adverse effects of high vitamin D intakes include *hypercalciuria* (excessive urinary calcium excretion) and *hypercalcaemia* (high concentration of calcium in blood). Excessive amounts of vitamin D are not normally available from dietary sources, and hence cases of vitamin D intoxication are rare. Nevertheless, toxicity may occur in individuals on excessive amounts of supplemented vitamins, for example, drinking milk fortified with inappropriately high levels of vitamin D_3. The signs and symptoms associated with it are anorexia, nausea and vomiting, followed by polyuria, polydipsia, weakness, nervousness and pruritis (itchiness). Renal function is impaired and metastatic calcifications may occur, particularly in the kidneys.

So, what should be the safe level of intake? For this, recommendations have been made. Let us read and find it out.

Recommended Dietary Allowances for Vitamin D

The recommendations of vitamin D are expressed as IU. 1 IU is defined as *the activity contained in 0.025 mcg of cholecalciferol*. EAR of 400 IU and on RDA of 600 IU is recommended by ICMR (2020) for all age groups, while emphasizing the importance of physical activity as a means of achieving adequate vitamin D status in a tropical country like India. Refer to Table 7.3.

The criterion for assessment of vitamin D status is given next.

Criteria for Assessment of Vitamin D Status

You may recall the events involved in calcium homeostasis described earlier in this section. We studied that sufficient 25-OH-D must be available to provide adequate steroid hormone *1,25-dihydroxycholecalciferol* ($1,25\text{-}(OH)_2D$) synthesis and hence an adequate level of plasma calcium. It becomes evident therefore, that vitamin D status can be assessed by measuring the circulating level of the steroid hormone *1,25-dihydroxycholecalciferol*. Plasma levels of 3-6 ng/dl is considered normal.

What is the effect of other nutrients on vitamin D metabolism and what are these nutrients? Let us find out next.

Interaction of Vitamin D with other Nutrients

Vitamin D metabolism is inter-related with calcium, phosphorous, vitamin K and iron. Let us discuss each of these:

- *Calcium*: The interaction of vitamin D with calcium has already been discussed earlier. Vitamin D initiates calcium absorption, as well as, activation of protein kinase.

Table 7.3 : EAR and RDA of vitamin D in case of minimal sun exposure (only with adequate dietary calcium intake)

	Body weight kg	EAR Unit/day	RDA Unit/day
Adult Men	65	400 IU	600 IU
Adult Women	55	400 IU	600 IU
Pregnant	-	400 IU	600 IU
Lactating	-	400 IU	600 IU
Infants (0-6mo)	5.8	-	400 IU
Infants (6-12mo)	8.5	-	400 IU
Children (1-3y)	11.7	400 IU	600 IU
Children (4-6y)	18.3	400 IU	600 IU
Children (7-9y)	25.3	400 IU	600 IU
Boys (10-12y)	34.9	400 IU	600 IU
Girls (10-12y)	36.4	400 IU	600 IU
Boys (13-15y)	50.5	400 IU	600 IU
Girls (13-15y)	49.6	400 IU	600 IU
Boys (16-18y)	64.4	400 IU	600 IU
Girls (16-18y)	55.7	400 IU	600 IU

Source: RDA and EAR- Nutrient Requirements for Indians, ICMR (2020), NIN

- *Phosphorous*: Vitamin D increases the activity of brush border alkaline phosphatase, which hydrolyzes phosphate ester bonds allowing phosphorous absorption.
- *Vitamin K*: An inter-relationship exists between vitamin D and K based on their relationship to the mineral calcium. Vitamin D has an impact on calcium metabolism and vitamin K-dependent proteins bind calcium. The two sites of action of vitamin D are bone and kidney tissues, where vitamin K-dependent calcium-binding proteins have been identified which regulate the production of crucial enzymes.
- *Iron*: A decrease in vitamin D has been observed as a result of iron deficiency.

With this, we end our study of vitamin D. Let us recapitulate what we have learnt so far by answering the questions given in check your progress exercise 2.

Check Your Progress Exercise 2

1) Fill in the blanks:

   a) The normal circulating plasma levels of vitamin D ranges from ..... to ..... ng/dl.

   b) ..... is vital to the utilization of calcium and phosphorous.

   c) Vitamin D deficiency leads to ..... in children and ..... in adults.

   d) Vitamin D is also referred to as ..... vitamin.

   e) ..... and ..... results in poor absorption of vitamin D.

2) Indicate whether the following statements are true or false. Also correct the false statements.

a) Vitamin D needs are met mostly from dietary sources.

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b) *Purdah system* increases availability of vitamin D.

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c) Craniotabes and rachitic rosary are associated with VAD.

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d) Cases of vitamin D intoxication are rare.

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e) The nutritional availability of vitamin D is less significant because it can be endogenously produced and retained for long periods by the tissues.

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3) Explain the synthesis of vitamin D_3 from its provitamin.

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4) List the symptoms of vitamin D deficiency in children.

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5) Explain the inter-relationship of vitamin D metabolism with vitamin K.

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Next, we take up the third fat soluble vitamin i.e. vitamin E.

7.5 VITAMIN E (ALPHA TOCOPHEROL)

Vitamin E is the generic term for *tocopherols* and *tocotrienols* that have a phenolic functional group on a chromane ring system with an isoprenoid side chain. Figure 7.11 illustrates the isoprene unit for your reference. *Isoprene*, as you can see, is a *branched chain unsaturated hydrocarbon of five carbon atoms* (C-C-C-C-C). This carbon skeleton forms the basis of carotenoids, steroids and tocopherols. The tocopherols and tocotrienols occur as homologues – α, β and γ – that differ in the number or location of methanyl substituents in the chromanol.

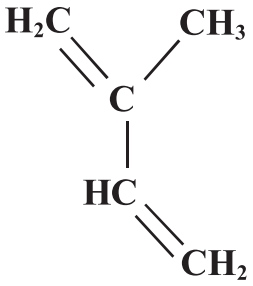


Figure 7.11: Isoprene unit

Having looked at the structure of tocopherols and tocotrienols, let us study about the food sources of vitamin E.

Sources and requirement of Vitamin E

Vitamin E is present in almost all foodstuffs. It is found in wheat germ, corn, nuts, seeds, olives, spinach, asparagus and other green leafy vegetables and vegetable oils like groundnut, soy, cotton seed and safflower are rich sources. The vitamin E content of edible oils is usually proportional to the amount of polyunsaturated fatty acid content of the oils. Table 7.4 gives the vitamin E content of some vegetable oils (mg tocopherol/100 g).

Table 7.4: Vitamin E content of vegetable oils (mg tocopherol/100 g)

Oil	α-tocopherol	γ-tocopherol	δ-tocopherol	α-tocotrienol
Coconut	0.5	0	0.06	0.5
Maize (corn)	11.2	60.2	1.8	0
Palm	25.6	31.6	7.0	14.3
Olive	5.1	Trace	0	0
Peanut	13.0	21.4	2.1	0
Soybean	10.1	59.3	26.4	0
Wheat germ	133.0	26.0	27.1	2.6
Sunflower	48.7	5.1	0.8	0

Source: Vitamin and mineral requirements in human nutrition, FAO/WHO 2004.

We may need to understand here that there may be a variation in the α-tocopherol levels or intake, the variation being ascribed mainly to the type and quantity of dietary oils used and the proportion of the different homologues in the oils (as highlighted in Table 7.4). For example, sunflower seed oil contains approximately 50 mg α-tocopherol/100 g in contrast to soybean oil that contains only 10 mg/100 ml.

Because vitamin E is naturally present in plant-based diets (whole grain cereals, dark green leafy vegetables, pulses, nuts and oilseeds as highlighted in Figure 7.12) and

animal products (such as egg yolk, butter and liver) and is often added by manufacturers to vegetable oils and processed foods, intakes are probably adequate to avoid overt deficiency in most situations. Exceptions may be during ecologic disasters and cultural conflicts resulting in food deprivation and famine.

So we have a large variety of foods which can help us meet our vitamin E needs. Next, once inside the body, what is the fate of vitamin E? Let us find out next.

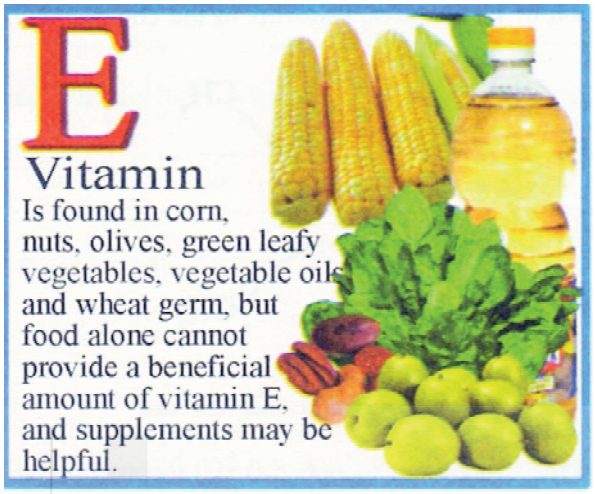


Figure 7.12: Food sources of vitamin E

The suggested Vitamin E requirement is 0.8 mg/g of dietary essential fatty acid. This roughly workes out to 8-10 mg tocopherol/d, depending on the edible oil used.

Absorption and Storage of Vitamin E

- *Absorption and Transport*

Absorption of vitamin E from the intestine depends on adequate pancreatic function, biliary secretion and micelle formation. Conditions for absorption are like those for dietary lipid, that is, efficient emulsification, solubilization within mixed bile salt micelles, uptake by enterocytes, and secretion into the circulation via the lymphatic system. Emulsification takes place initially in the stomach and then in the small intestine in the presence of pancreatic and biliary secretions. The resulting mixed micelle aggregates the vitamin E molecules, solubilizes the vitamin E, and then transports it to the brush border membrane of the enterocyte, probably by passive diffusion.Within the enterocyte, tocopherol is incorporated into chylomicrons and secreted into the intracellular space and lymphatic system and subsequently into the blood stream. Tocopherol esters, present in processed foods and vitamin supplements, must be hydrolyzed in the small intestine before absorption.

Vitamin E is transported in the blood by the plasma lipoproteins and erythrocytes. Chylomicrons carry tocopherol from the enterocyte to the liver, where they are incorporated into parenchymal cells as chylomicron remnants. The catabolism of chylomicrons takes place in the systemic circulation through the action of cellular lipoprotein, lipase. During this process, tocopherol can be transferred to high-density lipoproteins (HDLs). The tocopherol in HDLs can transfer to other circulating lipoproteins, such as low-density lipoprotein (LDL) and very low-density lipoproteins (VLDLs). During the conversion of VLDL to LDL in the circulation, some α-tocopherol remains within the core lipids and is thus incorporated in LDL. Most α-tocopherol then enters the cells of peripheral tissues within the intact lipoprotein through the LDL receptor pathway, although some may be taken up by membrane binding sites recognizing apolipoprotein (A-I and A-II) present on HDL.

Although the process of absorption of all the tocopherol homologues in the diet is similar, the α-form predominates in blood and tissue. From a nutritional perspective,

the most important form of vitamin E is α-tocopherol. It is absorbed faster and retained better than other forms. This is due to the action of binding proteins that preferentially select the α form over other forms.

- *Storage*

Vitamin E is mainly stored in muscles and adipose tissue. Vitamin E content of erythrocytes is about 20 percent of that in plasma and there is an efficient exchange between these pools. The vitamin is most concentrated in cellular fractions that are rich in membrane lipids such as mitochondria.

- *Elimination*

The primary oxidation product of α-tocopherol is *α-tocopheryl quinone* that can be conjugated to yield the glucuronate. This glucuronide is excreted in the bile as such or further degraded in the kidneys to α-tocopheronic acid glucuronide and hence excreted in the bile. Those vitamin E homologues not preferentially selected by the hepatic binding proteins are eliminated during the process of nascent VLDL secretion in the liver and probably excreted via the bile. Some vitamin E may also be excreted via skin sebaceous glands.

Next, let us now move on to the functions of vitamin E.

Functions of Vitamin E

Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defense system and is exclusively obtained from the diet. The main role of vitamin E and the biological activity of tocopherols are due to its antioxidant property. This antioxidant property of vitamin E is useful for various body processes and substances which is enumerated herewith.

1) *Protection of poly unsaturated fatty acids (PUFA) from oxidative damage*: The major biological role of vitamin E is to protect PUFAs and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals. Vitamin E is located primarily within the phospholipid bilayer of cell membranes. It is particularly effective in preventing lipid peroxidation – a series of chemical reactions involving the oxidative deterioration of PUFAs, as described in Figure 7.13. Elevated levels of lipid peroxidation products are associated with numerous diseases and clinical conditions.

   Let us understand the reactions involved in the oxidative deterioration of PUFAs next. The PUFAs, you may recall studying in the Nutritional Biochemistry

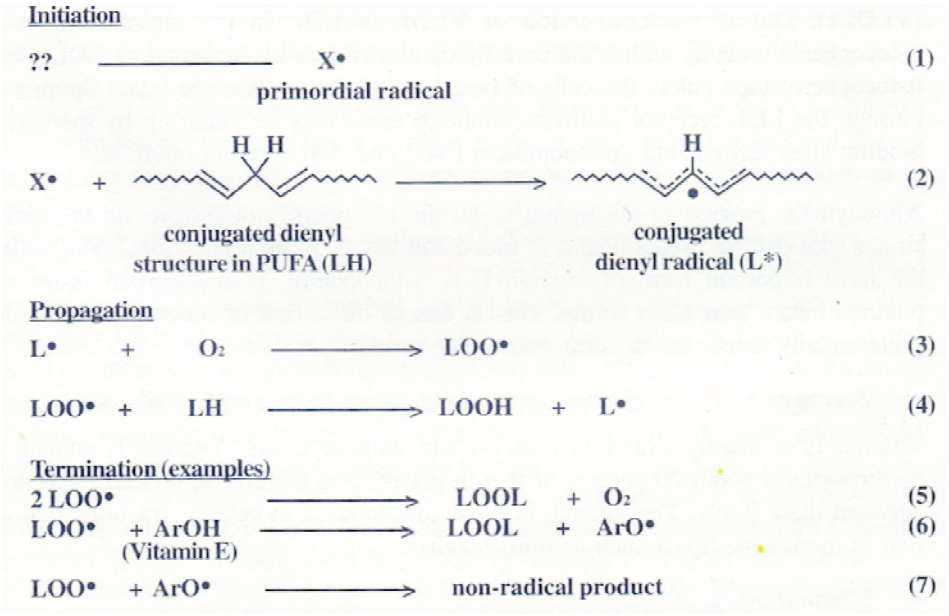


Figure 7.13: The autoxidation of PUFAs

Course, have methylene (-CH_2-) groups located between two double bonds. This type of functionality makes PUFA particularly sensitive to the flameless oxidation in air that is called *autoxidation*, because the doubly allelic hydrogen atoms in these methylene carbons are very rapidly abstracted by peroxyl radicals. Free radicals are produced in normal metabolism and by the effects of toxins on tissues.

These free radicals (eg. superoxide produced endogenously by many processes like phagocytosis) can cause the formation of lipid free radicals as shown in the initiation sequence reactions 1 and 2 in Figure 7.13 In reaction 2, initiation produces the lipid free radicals and a conjugated dienyl radical (L). The lipid radical L then undergoes the propagation sequence reaction 3 and 4 leading to the formation of lipid hydroperoxides, LOOH.

Lipid hydroperoxides produced in autoxidation processes are also formed in enzyme-catalyzed reactions. For example, the enzymes in cyclooxygenase pathway which converts arachidonic acid to several types of hydroperoxides. These have potent biological properties.

Autoxidation is a chain process. Reactions 3 and 4 continue to alternate until a termination occurs in which two radicals combine to form a new two-electron bond. When vitamin E is absent, from five to twenty five LOOH molecules are formed in reaction 4 for each primordial X radical formed in reaction 1. If vitamin E is present, it traps peroxylradicals to give stable LOOH molecule and a vitamin E radical (Ar O^0 in reaction 6). The vitamin E radical is stable enough to seek another LOO* radical as in reaction 7 and complete the termination sequence. In absence of vitamin E, however, each primordial radical produces <1 molecule LOOH. Thus, vitamin E effectively stops the autoxidation chain reaction which converts PUFA to lipid hydroperoxides, LOOH. One molecule of tocopherol can effectively protect 100 or more PUFA molecules from autoxidative damage. Biological membranes generally contain ~ one percent as many molecules of vitamin E as molecules of PUFA.

2) *Protection of erythrocytes*: Vitamin E protects erythrocytes from haemolysis by the production of oxidizing agents e.g. dialuric acid and hydrogen peroxide.

3) *Protection of cell membrane*: It protects the cell membrane from getting damaged from naturally occurring peroxides and toxic free radicals formed from fatty acids and oxidative tissue damage as described above.

4) *Protection against poisoning*: It protects liver from injury due to carbon tetra chloride poisoning.

5) *Protection of both vitamin A and carotene*: It protects vitamin A and β-carotene from destruction by oxidation, especially in the alimentary tract, thus sparing the supply of vitamin A available to the body.

6) *Synthesis of enzymes and proteins*: It serves as a co-repressor in the synthesis of certain enzymes and plays a specific role in the synthesis of haem proteins.

7) *Protection of mitochondria*: It protects the mitochondrial function of the muscles and cardiac tissue. Tocopherol acts as an electron acceptor in the electron transport system and prevents the disruption of mitochondria.

8) *Reduction in free radical generation*: Vitamin E acts synergistically with selenium thereby reducing susceptibility of LDL to oxidation, free radical generation and membrane damage. This antioxidant role of vitamin E together with selenium protects against cardiovascular diseases especially atherosclerotic lesions.

9) *Regulation of the enzyme activities*: Vitamin E regulates the activity of enzymes, δ-*amino levulinic acid* (*ALA*) *synthetase* in bone marrow and ALA *dehydrase* in liver.

10) *Prevention of diseases*: Because of its anti-oxidant function and its role in inhibiting cell proliferation of smooth muscles, vitamin E can be used for prevention/ treatment of diseases. Epidemiological studies suggest that dietary vitamin E influences the risk of cardiovascular disease. It has also been suggested that vitamin E supplementation (200-400 mg/day) may be appropriate therapeutically to moderate some aspects of degenerative diseases such as Parkinson disease, reduce the severity of neurologic disorders such as *tardive dyskinesia* (petentially irreversible and involuntory movements), prevent periventricular haemorrhage in pre-term babies, reduce tissue injury arising from ischaemia and reperfusion during surgery, delay cataract development, and improve mobility in arthritis sufferers.

From our discussion above, it must be clear that the major role of vitamin E lies in its antioxidant property. Next, let us get to know about the bioavailability of the tocopherols and the resulting diseases.

Bioavailability

For dietary purposes, vitamin E activity is expressed as *α-tocopherol equivalents* (α-TEs). One α-TE is the activity of 1mg α-tocopherol. To estimate the α-TE of a mixed diet containing natural forms of vitamin E, the number of milligrams of β-tocopherol should be multiplied by 0.5, γ-tocopherol by 0.1, and α-tocotrienol by 0.3.

The bioavailability of tocopherols varies inversely with the uptake. This means that ingesting four times the amount of the vitamin raises tissue levels by only two-fold. A high correlation exists between the total fat and tocopherol concentrations in blood serum. Thus, diseases associated with high serum lipids (hypothyroidism, diabetes, hypercholesterolemia) produce high plasma vitamin E levels and those associated to low serum lipids (abetalipoproteinemia-a genetic disorder that interferes with the normal absorption of fats and fat-soluble vitamins, malnutrition, cystic fibrosis) produce low vitamin E levels.

What then are the consequences of deficiency and excessive intake of vitamin E? Read the next sub-section and find out.

Deficiency and Toxicity of Vitamin E

Fortunately, vitamin E deficiency in human is extremely rare. This may probably be due to its wide occurrence in natural foods as highlighted above. Evidence of deficiency is however seen in individuals with chronic fat malabsorption e.g. sprue and fibrocystic disease of pancreas. Changes occurring in severe deficiency include disorders of reproduction, abnormalities of muscle, liver, bone marrow and brain function, defective embryogenesis, increased haemolysis of red blood cells, creatinuria and deposition of brownish ceroid pigment in smooth muscle. Skeletal muscle dystrophy may occur and, in certain species, is accompanied by cardiomyopathy.

Recent evidence has established that vitamin E deficiency is a cause of the impaired neuromuscular function, sometimes seen in patients with disorders that interfere with absorption or transport of the vitamin. Symptoms include poor reflexes, impaired locomotion, decreased sensation in the hands and feet, and changes in the retina. Disorders provoked by traces of peroxidized PUFAs in the diets of animals with low vitamin E status include cardiac or skeletal myopathies, neuropathies and liver necrosis. Muscle and neurological problems are also a consequence of human vitamin E deficiency. Early diagnostic signs of deficiency include leakage of muscle enzymes such as *creatine kinase* and *pyruvate kinase* into plasma, increased levels of lipid peroxidation products in plasma and increased erythrocyte haemolysis.

We read about the consequences of low intake of vitamin E on the human body. A very high intake of vitamin E can also elicit severe adverse reactions, as described next.

Toxicity

Vitamin E is relatively non-toxic. Adults tolerate doses as high as 100 to 1,000 IU per day. However, adverse effects such as muscle weakness, fatigue, nausea, diarrhoea, double vision, elevation of serum lipids, impaired blood coagulation and reduction of serum thyroid hormones occur due to indiscriminate ingestion of excessive amounts of vitamin E over long periods of time.

Evidence of pro-oxidant damage has been associated with the feeding of supplements but usually only at very high doses (e.g. >1000 mg / day).

Next, let us get to know about the requirements for vitamin E.

Recommended Dietary Allowance of Vitamin E

The requirements for the vitamin E are expressed in terms of tocopherol equivalents (TE). The suggested requirement of alpha tocopherol is 0.8 mg/g of dietary essential fatty acid.

This works out to 8-10mg tocopherol/d depending on the edible oil used vegetable oils and invisible fat of cereals and other foods like nuts and vegetables contribute to adequate tocopheorl content in Indian diet.

The criterion for assessment of vitamin E status in our body is presented, next.

Criteria for Assessment of Vitamin Status

Vitamin E is assessed by determining the plasma lipid fraction levels. 0.8 mg of total tocopherol/g total plasma lipids indicates adequate nutritional status.

The interaction of vitamin E with other nutrients is enumerated finally.

Interaction with other Nutrients

Vitamin E is directly related to selenium, other fat-soluble vitamins and PUFA. Let us study this relationship.

- *Selenium*: An inter-relationship exists between vitamin E and selenium, as selenium functions as an integral part of *glutathione peroxidase* – an enzyme – that converts lipid peroxide into a lipid alcohol.
- *Vitamins A, D and K*: A high intake of vitamin E interferes with the functions of other fat-soluble vitamins such as vitamin K absorption and vitamin D in terms of bone mineralization while in case of vitamin A deficiency. It lowers the rate of vitamin A depletion from the liver. It protects the cleavage products and substrate from oxidation. However, large doses of vitamin E inhibit β-carotene absorption or conversion to retinol in the intestine.
- *Polyunsaturated Fatty Acid (PUFA)*: A relationship between vitamin E and dietary PUFA is strong, as we have already studied earlier, because the requirement for the vitamin increases or decreases as the dietary intake of PUFA rises or falls.

We now end our study about vitamin E. Do answer the questions given in check your progress exercise 3 and check your understanding on the topic.

Check Your Progress Exercise 3

1) State whether the following statements are true or false. Also correct the false statements.

   a) Vitamin E is present in almost all food stuffs.

   .....

   .....

b) Tocopherols and tocotrienols have a phenolic functional group on a chromane ring system.

.....

c) Vitamin E supplementation can adversely affect insulin action.

.....

d) The vitamin E content of edible oils is usually proportional to the amount of polyunsaturated fatty acid content of the oils.

.....

2) Name a few rich sources of vitamin E.

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3) List the crucial functions of vitamin E.

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4) What is the recommended allowance for vitamin E? Name the unit in which the recommendations are expressed?

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5) Explain the interaction of vitamin E with other fat-soluble vitamins.

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The last vitamin covered in this unit is vitamin K. Vitamin K you must have read with relevance to our blood clotting mechanism. So let us get to know this vitamin in detail.

7.6 VITAMIN K

Vitamin K is an essential fat-soluble micronutrient; the only unequivocal role in health is in the maintenance of normal coagulation.

Vitamin K is the family name for a series of fat-soluble compounds which have a common 2-methyl-1,4-naphthoquinone nucleus (refer back to the Figure 7.1) but

differ in the structures of a side chain at the 3-position. They are synthesized by plants and bacteria. In plants, the only important molecular form is *phylloquinone* (vitamin K_1), which has a phytyl side chain. Bacteria synthesize a family of compounds called *menaquinones* (vitamin K_2), which have side chains based on repeating unsaturated 5-carbon (prenyl) units. Look up Unit 3 in the Nutritional Biochemistry Course to study the structure of these two compounds.

Menaquinones are designated menaquinone-n (MK-n) according to the number (n) of prenyl units. The compound 2-methyl-1,4-naphthoquinone (common name menadione) may be regarded as a provitamin because vertebrates can convert it to MK-4 by adding a 4-prenyl side chain at the 3-position.

What are the food sources of vitamin K? Let us find out.

Sources of Vitamin K

As mentioned above, in plants, the only important molecular form of vitamin K is *phylloquinone.* Phylloquinone is distributed ubiquitously throughout the diet, and the range of concentrations in different food categories is very wide. In general, the highest values (normally in the range 400-700 mg / 100 g) are found in green leafy vegetables (such as spinach, cauliflower, cabbage and lettuce as shown in Figure 7.14). The next best sources are certain vegetable oils (e.g. soybean, rapeseed and olive), which contain 50-200 mg/100g, other vegetable oils, such as peanut, corn, sunflower and safflower, however, contain much lower amounts of phylloquinone (1-10 mg/100 g). The great differences between vegetable oils with respect to vitamin K content obviously present problems for calculating the phylloquinone contents of oil containing foods when the type of oil is not known. Other good sources include animal foods such as egg yolk, milk and organ meats like liver.



Figure 7.14: Food sources of vitamin K

Importance of Intestinal Bacterial Synthesis as a Source of Vitamin K

Intestinal microflora synthesize large amounts of menaquinones, which are potentially available as a source of vitamin K. Quantitative measurements at different sites of the human intestine have demonstrated that most of these menaquinones are present in the distal colon. Major forms produced are MK-10 and MK-11 by *Bacteroides*, MK-8 by *Enterobacter*, MK-7 by *Veillonella*, and MK-6 by *Eubacterium lentum etc.*

However, the balance of evidence suggests that the bioavailability of bacterial menaquinones is poor because they are for the most part tightly bound to the bacterial cytoplasmic membrane and also because the largest pool is present in the colon, which lacks bile salts for their solubilization.

What about the bioavailability of vitamin K from different foods? Let us read the next sub-section and find out.

Bioavailability of Vitamin K

Very little is known about the bioavailability of the K vitamins from different foods. It has been estimated that the efficiency of absorption of phylloquinone from boiled spinach (eaten with butter) is no greater than 10% compared with an estimated 80% when phylloquinone is given in its free form. This poor absorption of phylloquinone from green leafy vegetables may be explained by its location in chloroplasts (organelles in plant cells that conduct photosynthesis) and tight association with the thylakoid membrane (a phospholipid bilayer memberane-bound compartment internal to chloroplasts). In comparison, the bioavailability of MK-4 from butter artificially enriched with this vitamin was more than two-fold higher than that of phylloquinone from spinach. The poor extraction of phylloquinone from leafy vegetables, which as a category represents the single greatest food source of phylloquinone, may place a different perspective on the relative importance of other foods with lower concentrations of phylloquinone (e.g. those containing soybean and rapeseed oils) but in which the vitamin is not tightly bound and its bioavailability is likely to be greater.

Vitamin K availability varies directly with fat intake and any condition of fat malabsorption reduces its bioavailability. In healthy adults, absorption of phylloquinone has been estimated to be 80 percent when phylloquinone is administered in its free form as discussed above, but decreases significantly when absorbed from foods. Cooking has no effect, but addition of fat increases absorption multifold.

What happens to the vitamin in our body? Let us find out next.

Absorption, Storage and Elimination of Vitamin K

Dietary vitamin K, mainly phylloquinone, is absorbed chemically unchanged from the proximal intestine after solubilization into mixed micelles composed of bile salts and the products of pancreatic lipolysis. In healthy adults, the efficiency of absorption of phylloquinone in its free form is about 80%. Within the intestinal mucosa, the vitamin is incorporated into chylomicrons, is secreted into the lymph, and enters the blood via the lacteals (minute intestinal lymph-carrying vessels). Once in the circulation, phylloquinone is rapidly cleared at a rate consistent with its continuing association with chylomicrons and the chylomicron remnants, which are produced by lipoprotein lipase hydrolysis at the surface of capillary endothelial cells. Although phylloquinone is the major circulating form of vitamin K, MK-7 is also present in plasma, at lower concentrations and with a lipoprotein distribution similar to phylloquinone.

Vitamin K is stored in the liver – the site of synthesis of coagulation proteins – and consists of 90 percent menaquinones. Phylloquinones and menaquinones are also found in extra hepatic tissues. Phylloquinone levels are high in liver, heart and pancreas.

Vitamin K is extensively metabolized in the liver and excreted in the urine and bile. About 60-70% of the amount of phylloquinone absorbed from each meal will ultimately be lost to the body by excretion. This, therefore, suggests that the body stores of phylloquinone are being constantly replenished.

Next, let us get to know the functions of vitamin K in the body.

Functions of Vitamin K

The functions of vitamin K are both physiological and biochemical. These include:

1) *Blood coagulation*: The primary function of vitamin K in the body is in the maintenance of normal blood coagulation. The vitamin K-dependent coagulation proteins are synthesized in the liver and comprise Factor II (prothrombin), Factor VII (proconvertin), Factor IX (Christmas factor) and Factor X (Stuart factor), which have a haemostatic role i.e. they are procoagulants that arrest and prevent bleeding. Let us get to know how this mechnism works. Prothrombin is converted

to its active form, *thrombin*, which in turn, is necessary for the formation of *fibrin*, a protein that is the basis for a blood clot, as shown in Figure 7.15. Vitamin K also acts as a cofactor for an enzyme in the liver which converts glutamic acid residues in a precursor process to gamma-carboxy glutamic acid. This reaction is necessary before prothrombin can function in blood coagulation.

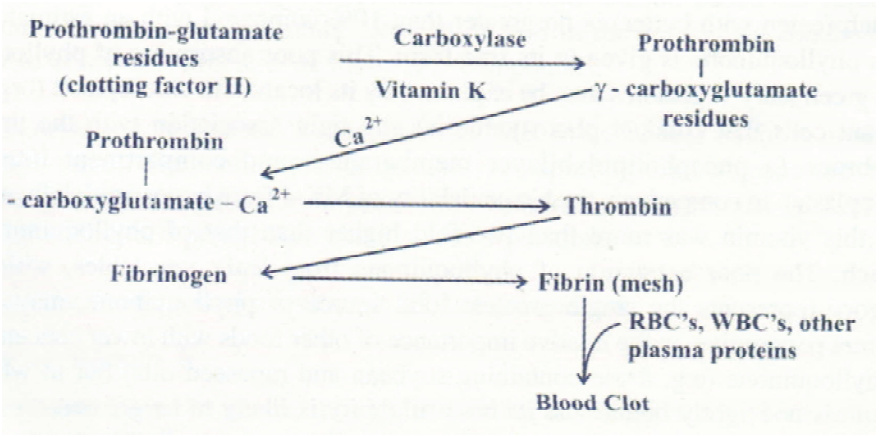


Figure 7.15: Biological role of vitamin K in the formation of blood clot

2) *Vitamin K-dependent carboxylation*: Vitamin K acts as a cofactor in the synthesis of γ-carboxyglutamic acid (Gla) from glutamic acid residues required for the normal coagulation of blood. This is well explained in the Nutritional Biochemistry Course in Unit 10, section 10.3, sub-section 10.3.4 under the vitamin K cycle. Figure 7.16 here also illustrates the vitamin K cycle.

The biological role of vitamin K, therefore, is to act as a cofactor for a specific carboxylation reaction that transforms selective glutamate (Glu) residues to γ-carboxyglutamate (Gla) residues. The reaction is catalyzed by a microsomal enzyme, *γ-glutamyl, or vitamin K-dependent carboxylase*, which in turn is linked to a cyclic salvage pathway known as the *vitamin K epoxide cycle*. The hydroquinone (reduced form of vitamin K), CO_2 and O_2 are required for the reaction. During the catalysis, hydroquinone is oxidized to vitamin K 2,3-epoxide and the energy derived from the oxidation drives the carboxylation.

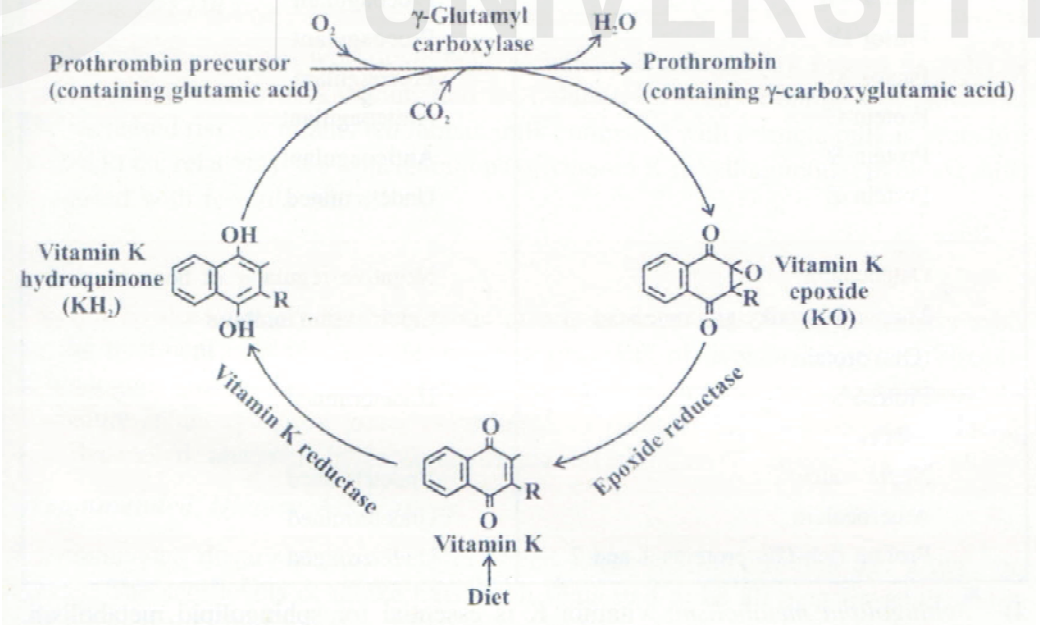


Figure 7.16: Vitamin K cycle

3) *Vitamin K dependent proteins*: The four vitamin K-dependent procoagulants (factor II or prothrombin, and factors VII, IX, and X), about which we studied

above, are *serine proteases* that are synthesized in the liver and then secreted into the circulation as inactive forms (zymogens). Their biological activity depends on their normal complement of Gla residues, which are efficient chelators of calcium ions. In the presence of Gla residues and calcium ions, these proteins bind to the surface membrane phospholipids of platelets and endothelial cells where, together with other cofactors, they form membrane-bound enzyme complexes. When coagulation is initiated, the zymogens of the four vitamin K-dependent clotting factors are cleaved to yield the active protease clotting factors.

Two other vitamin K-dependent proteins, *protein C* and *protein S*, play a regulatory role in the inhibition of coagulation. The function of protein C is to degrade phospholipid-bound activated factors V and VIII in the presence of calcium. Protein S acts as a synergistic cofactor to protein C by enhancing the binding of activated protein C to negatively charged phospholipids. There is an evidence that protein S is synthesized by several tissues including the blood vessel wall and bone and may have other functions besides its well-established role as a coagulation inhibitor. Yet another vitamin K-dependent plasma protein (protein Z) is suspected to have a haemostatic role but its function is currently unknown.

Apart from the coagulation proteins, several other vitamin K-dependent proteins have been isolated from bone, cartilage, kidney, lungs and other tissues. Only two*, osteocalcin* and *matrix Gla protein* (MGP), have been well characterized. Both are found in bone but MGP also occurs in cartilage, blood vessel walls, and other soft tissues. One function of MGP is to inhibit mineralization. Thus far, no clear biological role for osteocalcin has been established despite its being the major noncollagenous bone protein synthesized by osteoblasts. Nephrocalcin has been isolated from kidney and urine. Atherocalcin, plaque Gla protein, proline rich Gla proteins have been identified from atheromatous plaques, spinal and thyroid tissues. Table 7.5 shows the vitamin K dependent proteins and their functions.

Table 7.5: Vitamin K dependent proteins and their functions

Proteins	Physiological Function
Blood coagulation	
Prothrombin	Procoagulant
Factor VII	Procoagulant
Factor IX	Procoagulant
Factor X	Procoagulant
Protein C	Anticoagulant
Protein S	Anticoagulant
Protein Z	Undetermined
Bone	
Osteocalcin	Negative regulator of bone formation
Matrix γ-carboxy glutamic acid (Gla) protein	Calcification inhibitor
Protein S	Undetermined
Others	
Nephrocalcin	Undetermined
Atherocalcin	Undetermined
Proline rich Gla proteins 1 and 2	Undetermined

4) *Sphingolipid metabolism*: Vitamin K is essential for sphingolipid metabolism. Spingolipids, as you would recall from your Biochemistry Course, are a class of *membrane lipids that* are composed of one molecule of the long-chain amino alcohol sphingosine (4-sphingenine) or one of its derivatives, one molecule of a long-chain acid, a polar head alcohol and sometimes phosphoric acid in diester linkage at the polar head group.

5) *Prevents bone loss*: Vitamin K is known to inhibit bone loss through inhibiting effect on osteoclast formation.

Thus, adequate levels of vitamin K must be maintained in the human body. From functions, we move on to study about the deficiency and toxicity of vitamin K.

Deficiency and Toxicity of Vitamin K

Both excess and low intakes can have serious implications on human health. Although such conditions arise rarely, these can often be life-threatening. Our subsequent discussions are pertaining to excess and deficient intake.

Adults are usually protected from a lack of vitamin K because vitamin K is widely distributed in plant and animal tissues, the vitamin K cycle conserves the vitamin, and microbiological flora of the normal gut synthesizes menaquinones. Also, a normal diet contains about 300 to 500 mcg vitamin K daily and therefore supplies at least three times the amount of recommended vitamin K.

Vitamin K deficiency leads to a lowered prothrombin level and increased clotting time, and thereby haemorrhages. The factors that lead to vitamin K deficiency include:

1) Marginal dietary intake if one undergoes trauma and extensive surgery etc.
2) Inadequate intake of vitamin K by the mother leads to haemorrhagic disease in the newborn, with low prothrombin level.
3) Inadequate intestinal absorption (disease of liver and intestine such as biliary obstruction, malabsorption and parenchymal liver disease) leads to deficiency in adults. Large amounts of vitamin A and E may interfere with the absorption or metabolism of vitamin K. In severe disease of the liver, the synthesis of the clotting factors is impaired even though the source of vitamin K is adequate.

The population groups that appear most at risk for vitamin K deficiency are newborn infants and people who have been injured and have renal insufficiency.

In infants up to around age 6 months, vitamin K deficiency, although rare, represents a significant public health problem throughout the world. The deficiency syndrome is traditionally known as *haemorrhagic disease of the newborn*. More recently, in order to give a better definition of the cause, it has been termed *vitamin K deficiency bleeding* (*VKDB*).

Epidemiological studies worldwide have identified two major risk factors for VKDB viz; exclusive human-milk feeding and the failure to give any vitamin K prophylaxis. The increased risk for infants fed human milk compared with formula milk is probably related to the relatively low concentrations of vitamin K (phylloquinone) in breast milk compared with formula milks.

Toxicity

Vitamin K does not produce any toxic effects in doses (10-20 mg) normally used for the treatment of subjects suffering from disorders of liver or intestines. Vitamin K analogues (menadione and water soluble forms of menadione), administered to premature infants produce toxicity attributed to increased breakdown of red blood cells (haemolytic anaemia), hyperbilirubinaemia and inhibition of glucouronide formation.

Recommended Dietary Allowances

Recommended dietary intakes have not been suggested for different age groups or gender. The safe levels of intake have been suggested to be 55 mcg of vitamin K/d for adults. The recommended nutrient intake for vitamin K, as suggested by ICMR (2020) and is in tune with recommendations of FAO/WHO, are presented in Table 7.6.

Table 7.6: Recommended nutrients intakes for vitamin K

Age Group	Recommended Nutrient Intake† µg /day
Infants and children	
0 - 6 months	5*
7 - 12 months	10
1 - 3 years	15
4 - 6 years	20
7 - 9 years	25
Adolescents, 10 - 18 years	
Females	35 - 55
Males	35 - 55
Adults	
Females, 19 - 65 years	55
65+ years	55
Males, 19 - 65 years	55
65+ years	55
Pregnancy	55
Lactation	55

† The RNI for each age group is based on a daily intake of approximately 1 microgram/body weight of phylloquinone.

* This intake cannot be met by infants who are exclusively breast-fed.

Having learnt about the requirements, let us next review the criteria for assessment of vitamin K status.

Criteria for Assessment of Vitamin K Status

The parameters of *blood clotting time* and *prothrombin time* are used as criteria to assess vitamin K status as this vitamin is vital for the formation of the factors involved in blood coagulation especially prothrombin. A normal prothrombin time is considered to be between 11 and 13 seconds, while a duration greater than 25 seconds is associated with major bleeding. In addition, maintenance of plasma prothrombin concentrations in the range 80-120 mg/ml suggests adequate vitamin K status.

Finally, let us get to know about the interaction of vitamin K with other nutrients.

Interaction with other Nutrients

Vitamin K absorption is inter-related to other fat-soluble vitamins (A and E) and calcium.

- *Vitamins A and E*: Excess vitamin A interferes with vitamin K absorption while the α-tocopherol or vitamin E, as we have already studied earlier, also affects absorption, function and metabolism of vitamin K. It may block the regeneration of the reduced form of vitamin K and/or may affect prothrombin formation.

- *Calcium*: Since vitamin D functions have an impact on calcium metabolism, an inter-relationship exists between vitamin K-dependent proteins and vitamin D, as well as, calcium. In the tissues of the bones and kidney, where vitamin D plays a major role, vitamin K-dependent calcium-binding proteins have been identified and shown to regulate the production of crucial enzymes.

With a discussion on vitamin K, we end our study on fat-soluble vitamins. The next unit will focus on the water-soluble vitamins.

Let us now perform the check your progress exercise 4 to assess our knowledge based on the above discussions.

Check Your Progress Exercise 4

1) State whether the following statements are true or false. Also correct the false statements.

   a) Half of the vitamin K needed by humans is manufactured in the intestinal tract.

   .....

   b) Vitamin K is stored in adipose tissues.

   .....

   c) The bioavailability of vitamin K depends on the status of fat in the body.

   .....

   d) Vitamin K is responsible for the formation of prothrombin.

   .....

2) Enumerate physiological and biochemical functions of vitamin K.

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   .....

3) List the factors that lead to vitamin K deficiency.

   .....

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   .....

   .....

4) Where are vitamin K and its forms stored in our body?

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   .....

5) Give normal standard values for prothrombin time which is used for assessing vitamin K status.

   .....

   .....

7.7 LET US SUM UP

This unit focussed on the fat-soluble vitamins. Fat-soluble vitamins are vital to health. They can be obtained from inexpensive, readily available plant foods and sunlight. A summary of the important functions and sources of fat-soluble vitamins is presented herewith. We learnt that the deficiency of vitamin A is a nutritional disorder of public health significance in India.

Summary of Fat Soluble Vitamins

Vitamins	Sources	Functions
Vitamin A	• Retinol: liver, egg yolk, cream, butter, ghee, milk • β-carotene: yellow and orange vegetables, green leafy vegetables	• Maintenance of health of epithelial tissues • Vision in dim light • Growth of skeletal and soft tissues • Resistance to infections
Vitamin D	• Action of sunlight on the skin • Animal foods like eggs, butter, fish liver oil	• Absorption of calcium and phosphorous • Deposition of calcium and phosphorous in bones
Vitamin E	• Vegetable oils, whole grains, deep green leafy vegetables, pulses, nuts and oilseeds.	• Protection of unsaturated fatty acids, vitamin A and C from destruction in the body/food.
Vitamin K	• Dark green leafy vegetables, egg yolk, liver • Bacterial synthesis	• Clotting of blood.

7.8 GLOSSARY

Abetalipoproteinemia : a rare inherited disorder of fat metabolism characterized by severe deficiency β-lipoproteins and abnormal RBCs and abnormally low cholesterol levels.

Arthralgias : neuralgic pain in a joint or joints.

Beta carotene : a fat-soluble carotenoid pigment present in plants which is a precursor of vitamin A.

Bioavailability : the fraction of ingested vitamins utilized for normal physiological functions and storage.

Blepharitis : an inflammation of the eyelid margins.

Chromanol ring system : the most important groups of substances in which the all-carbon ring is heterocyclic.

Coenzymes : specific substances which are essential for proper functioning of certain enzymes.

Cystic fibrosis : a condition in which there is section of abnormal mucus in lungs and problems with pancreatic function and food absorption.

Differentiation : the growth of cells into a specific type of cell.

Isoprene : a branched chain composed of unsaturated hydrocarbon of five carbon atoms.

Myalgias : pain in the muscles.

Osteocalcin : a protein responsible for the deposition of calcium salts in the bone.

Rhodopsin : a pigment formed by the combination of a specific form of vitamin A with a protein.

Seco-steroids : the steroids in which one of the rings have been broken.

Sprue : a chronic disorder that occurs in children and adults in which nutrients are not absorbed. Symptoms include

foul-smelling diarrhoea and emaciation.

Vitamins : organic compounds (other than carbohydrates, fats and protein) which are needed only in small amounts by the body.

7.9 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1) i) maintenance of visual perception
   ii) Retinol (alcohol) dehydrogenase
   iii) Vitamin A
   iv) Osteocalcin
   v) 350; 400
   vi) 12-25

2) Retinoids typically consist of 4 isoprenoid units joined head-to-tail with 5 conjugated carbon-carbon double bonds. Retinol can be oxidized reversible to retinal (vision cycle) and further to retinoic acid (growth and reproduction). The primary storage forms are retinyl esters. Retinoids are usually obtained from animals whereas, carotenoids are principally from plant origin.

3) Vitamin A plays most significant role in maintaining vision. The key component associated with vision is the photosensitive pigment of the eye, that is the visual purple or rhodopsin. It consists of the protein opsin bound to a pigment cis - isomer of retinene formed by the oxidation of retinol in the epithelium of the rods in the retina of the eye by alcohol dehydrogenase in the presence of NAD. The action of light bleaches the visual purple or dissociates rhodopsin to opsin and retinene. Resynthesis of rhodopsin is isomer specific i.e. it can be regenerated only after retinene is rearranged to the 11-cis form. The rearrangement is possible both as a photochemical reaction and an oxygen-dependent dark reaction.

4) Any of the five factors from the following:

   inhibition by intrinsic matrix, inhibition by dietary fiber sources, differential crowding by stereoisomeric forms, inverse relationship between ingested amount and uptake, intraluminal oxidative destruction, enhancement by presence of fat and oil, enhancement by cooking and processing, amount of provitamin A presented to the cell, differential conversion by stereoisomeric form, and host's underlying vitamin A status.

5) An intake above 1000,000 mg or 300,000 IU elicits signs of acute toxicity. It manifests as increased intracranial pressure and vomiting, which may lead to death unless ingestion is discontinued.

6) Clinical Assessment, Conjunctival Impression Cytology (CIC), Dietary Assessment Criteria, Serum vitamin A content, Liver biopsy assays, and Dark adaptation.

Check Your Progress Exercise 2

1) a) 3, 6
   b) Vitamin D
   c) Rickets and osteomalacia
   d) sunshine
   e) Sprue and celiac disease

2) a) False – met from sunshine

b) False – decreases exposure to sunlight

c) False – vitamin D deficiency

d) True

e) True

3) Vitamin D_3 is formed from its precursor 7-dehydrocholesterol. The synthesis of vitamin D_3 from its provitamin: 7-dehydrocholesterol occurs by UV irradiation and proceeds from the provitamin to the previtamin and finally to the vitamin.

4) The symptoms of rickets are:

- In case of young infants, delayed closure of the fontanelles i.e. a soft membranous gap between the cranial bones, softening and reduced mineralization of the skull (craniotabes).
- While in older infants, sitting and crawling are delayed and there is bossing of skull. Also there are soft, fragile bones, bow legs enlargement of the costochondral junction with rows of knobs or beads forming the Rachitic Rosary; pigeon chest and spinal curvature.

  Other symptoms include: enlargement of wrist, knee (knock-knees) and ankle joints; poorly developed muscles; lack of muscle tone; pot belly being the result of weakness of abdominal muscles; weakness with delayed walking; restlessness and nervous irritability; high serum alkaline phosphatase; low inorganic blood phosphorus; normal or low serum calcium. Tetany and delayed dentition and malformation of the teeth, permanent teeth more subject to decay may also be observed.

5) An inter-relationship exists between vitamin D and K based on their relationship to the mineral calcium. Vitamin D affects calcium metabolism and vitamin K-dependent proteins bind calcium. The two sites of action of vitamin D are bone and kidney tissues, where vitamin K-dependent calcium-binding proteins have been identified which regulate the production of crucial enzymes.

Check Your Progress Exercise 3

1) a) True

b) True

c) False, vitamin E improves insulin action

d) True

2) It is found in wheat germ, corn, nuts, seeds, olives, spinach, asparagus and other green leafy vegetables and vegetable oils like ground nut, soy, cotton seed and safflower.

3) Major functions include: Protection of poly unsaturated fatty acids (PUFA) from oxidative damage, protection of erythrocytes, cell membrane, mitochondria, vitamin A and carotene, synthesis of enzymes and proteins, protection against poisoning, maintenance of body processes, reduction in free radical generation, regulation of the enzyme activities, prevention of diseases.

4) The RDA for vitamin E is 8 mg for females and 10 mg for males. The requirements for the vitamin E are expressed in terms of tocopherol equivalents.

5) A high intake of vitamin E interferes with the functions of other fat-soluble vitamins such as vitamin K absorption and vitamin D in terms of bone mineralization while in case of vitamin A deficiency, it lowers the rate of vitamin A depletion from the liver. However, large doses of vitamin E inhibit β-carotene absorption or conversion to retinol in the intestine.

Check Your Progress Exercise 4

1) a) True

   b) False – liver

   c) True

   d) True

2) Blood coagulation, Vitamin K- dependent synthesis of γ- carboxyglutamic acid, functioning of vitamin K dependent proteins, sphingolipid metabolism, and prevents bone loss.

3) Marginal or inadequate dietary intake or inadequate intestinal absorption leads to a deficiency in adults, children and infants.

4) Vitamin K is stored in the liver of which 90 percent are menaquinones. Phylloquinones and menaquinones are also found in extra hepatic tissues. Phylloquinone levels are high in liver, heart and pancreas.

5) A prothrombin time between 11 and 13 seconds.

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