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Food Engineering

Most fortificated nutraceutical vitamins: standpoint from biochemistry, functionality, utilization strategies and medical genomics

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Abstract
Nutraceutical vitamins are micronutrients of foods and beverages that play an essential role in food science and technology. Most fortificated vitamin A, E, D and folic acid as antioxidative defence system components and that of with gene regulation functions have been considered. Up-to-date scientific overview of the biochemistry, functionality, utilization strategies and human genomics of above-mentioned most fortificated nutraceutical vitamins were emphasized

Keywords: Nutraceutical vitamins, vitamin A, E, D, Folic acid

CONTENT

1. Introduction

1. The unified introduction to food vitamins from a biochemical and human genomics standpoint

Vitamins are micronutrients of foods and beverages that play an essential role in human nutrition. The occurrence of the vitamins in the various groups of food is related to their water or fat-solubility. They comprise a diverse group of organic compounds that are nutritionally essential minor components.

Vitamin function *in vivo* in several ways, including (a) as coenzymes or their precursors (niacin, thiamin, riboflavin, vitamin B_6, vitamin B_{12}, biotin, pantothenic acid, and folate; (b) as components of the antioxidative defense system (ascorbic acid, certain carotenoids and vitamin E); (c) as factors involved in genetic regulation (vitamin A and vitamin D, vitamin B_6, folate) is precious for human health and nutrition (1,2) (Figure 1.1.1.).

Food is a very complex matrix made up of lots of many individual components including fat and water soluble vitamins that a group of essential organic molecules. Fat soluble (A,D,E,K) and water soluble vitamins (B group, C, biotin, pantothenic acid) in foods have been categorized as above-mentioned three groups from a biochemical standpoint (Figure 1.1.1.).

The organism must obtain from exogenous sources and that is essential for the organism's normal metabolic and physiological functions (3). The elimination of vitamins from the diet must result in a more-or-less clearly defined deficiency disease, and restoration must cure or prevent that deficiency disease.

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With developing of functional foods, nutraceuticals and dietary patterns, “dietary nutriome” (nutrient profile and composition) and genome health maintenance has been improved. Nutrigenomics based on genome healths is an emerging and recent important field of food nutritional science due to it is increasingly evident that optimal concentration of micronutrients for the prevention of genome damage is dependent upon genetic polymorphisms that alter the gene function involved directly or indirectly in DNA repair and metabolism. It has shown that above-average intake of certain micronutrients (i.e. vitamin E, retinol, folate, vitamin B_6) is associated with a reduced genome damage rate measured using the micronucleus assay (4).

In this chapter context, most fortificated vitamins as antioxidative defence system components and vitamins with gene regulation functions have been considered

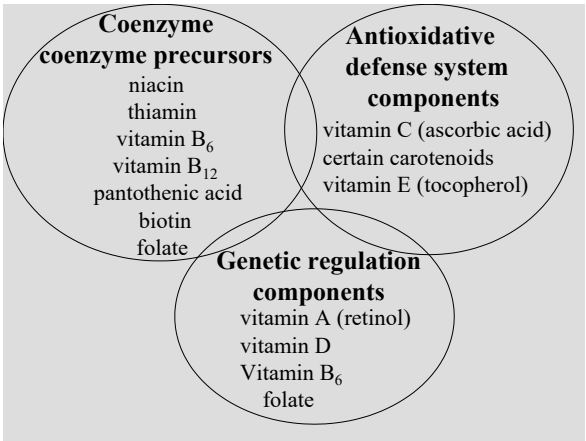

Figure 1.1.1. Vitamin function *in vivo*

2. Preliminaries

2. Up-to-date scientific overview of the most fortificated vitamins as antioxidative defense system components and as factors involved in genetic regulation

Foods and beverages are fortified with micronutrients and bioactive compounds to prevent deficiencies or to provide additional health effects (106). The first step in the establishment of a fortification process must be the determination of the extent and the magnitude of the vitamin deficiency, as veil as its distribution among the different ecological regions, socioeconomic levels and age groups.

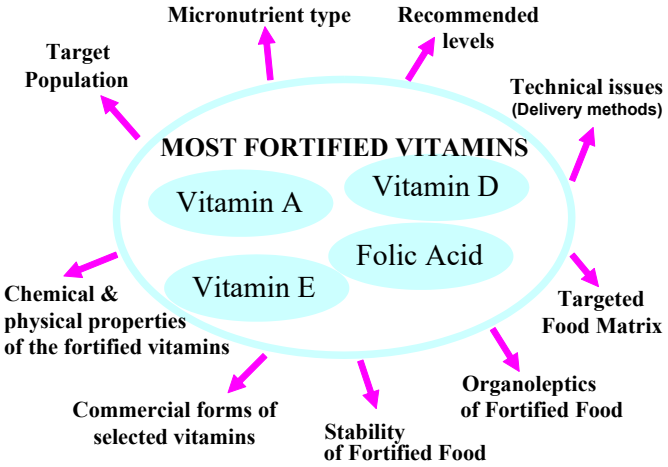

Figure 2.2.1. Possible Factors on Most Fortified Vitamins

It is reported that the most fortified vitamins are vitamin E, A, D and folic acid. Figure 2.2.1. shows the possible factors on most fortified vitamins for human nutrition. It is noteworthy that the target population, fortificated vitamin type as micronutrient, recommended levels of vitamins, technical issues such as vitamin delivering to targeted foods, chemical and physical properties of the fortified vitamins, commercial forms of selected vitamins, targeted food matrix, stability of fortified food, organoleptic quality of fortified food are extremely important for fortification strategy (Figure 2.2.1.).

Antioxidants quench free radicals via donating the phenolic H and an electron. They exhibit variable degrees of efficiency for protection of human cells. Vitamin E (tocopherols and tocotrienols), vitamin C (ascorbic acid) and certain carotenoids (β-carotene), when not esterified, have the ability to act as antioxidants (1). Recently, a great interest has been focused on antioxidant vitamins in foods, particularly owing to their

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likely role in the prevention of coronary heart diseases and cancer (5-7). Vitamins especially vitamin A and vitamin D and folate as factors involved in genetic regulation for human genomics.

Fortificated vitamins as micronutrient should be safe for human nutrition and tolerable upper intake levels (ULs) should be established. After fortification, the use of health claims on products is thought to result in better marketing (108).

The effect of fortification is both fast and broad. When a staple food which is consumed regularly by the majority of the population is utilized as a fortification vehicle, high population coverage can be easily accomplished. The combinations of micronutrients increase the cost-effectiveness of fortification (108).

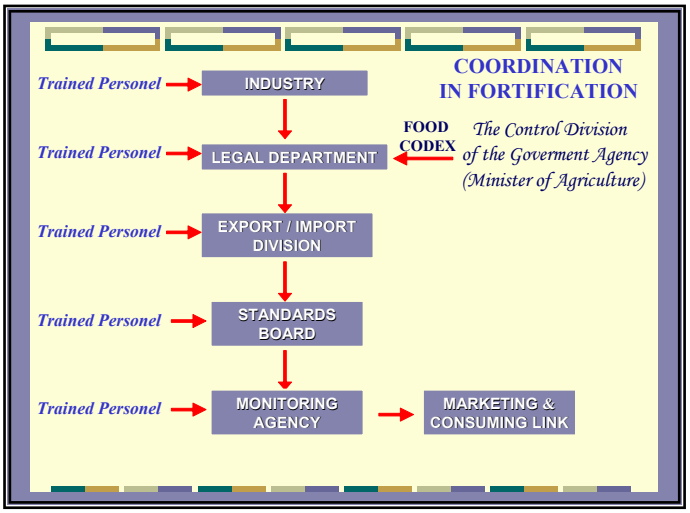

Figure 8.2.2. The Coordination Strategies in Fortification (Source: Adopted from Tokuşoğlu, 2006. *Fortification Criteria of Vegetable Oils and Margarines with Alpha-Tocopherol and Tocopheryl Acetate: The Regulations on Vitamin E Labeling*. Lecture. In World Conference and Exhibition on Oilseed and Vegetable Oil Utilization, Processing, By-Products, Biodiesel, Specialty and Functional Oils, and New Applications & Technologies 14-16 August 2006, Istanbul)

Figure 2.2.2. demonstrates the coordination strategies in fortification. Prior to Food codex by The Control Division of the Goverment Agency (generally Minister of Agriculture) are received to legal department, industrial manufacturing of fortificated foods are performed. Then fortification with trained personel is controlled export-import division, standard boards, monitoring agency, respectively and evaluated via marketing and consuming link (107).

The biochemistry, functionality and dietary reference intakes (DRI) of most fortificated vitamins as antioxidative defense system components vitamin E, certain carotenoids and as factors involved in genetic regulation components vitamin A, vitamin D and folic acid were focused on.

2.2.1. The Biochemistry, Functionality and Dietary Reference Intakes of Antioxidant Vitamin E

Vitamin E is generic term that represents four tocopherol and four tocotrienols of varying biological potency. "Tocopherol" term correctly refers to the methyl-substituted derivatives of tocol (8) (Figure 2.2.1.1.).

	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

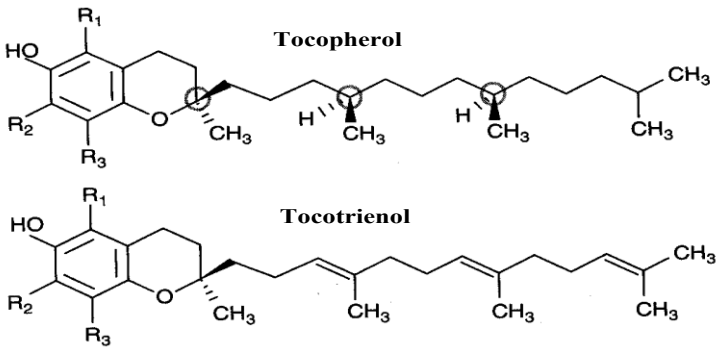

Figure 8.2.1.1. Vitamin E structures (8)

Tocopherols occur widely in nature and are monophenolic antioxidants that are composed of eight different compounds belonging to two families, namely tocols and tocotrienols, referred to as α, β, γ or δ, depending on the number and the position of methyl groups of attached to the chromane rings (8.9) (Figure 2.2.)

Vitamin E is a generic term which includes, in decreasing order of physiological activity alfa (α-), beta (β-), gamma (γ-) ,and delta (δ-) tocopherols (α-T, β-T, γ-T, δ-T) (2). The most biologically active is α-tocopherol, but β-, γ-, and δ-tocopherols also have important biologic activity (13).

The major lipophilic antioxidant is vitamin E especially α-tocopherol, that inhibits peroxidation of polyunsaturated fatty acids (PUFAs) in cell membranes and it is a liposoluble vitamin with an antioxidant capacity, reacting with peroxy radicals and other free radicals (22). Vitamin E that is a potent peroxyl radical scavenger and especially protects PUFA within phospholipids of cellular biological membranes and in plasma proteins. All vitamin E forms act as lipid soluble chain-breaking antioxidants and prevent the oxidation of fats and vitamin A (8,9).

The biological activity of vitamin E is thought to reflect its ability to quench oxygen- and carbon-based free radicals and thus to protect the organism from oxidative damage. Vitamin E is a dietary lipid that is essential for vertebrate health and fertility (10,13).

Epidemiological and numerous studies indicated that diet including tocopherols prevent the risk of cardiovascular diseases and cancer (16-18,21). Tocopherols has also been associated with a reduction in the risk of disorders connected to free radicals, such as atherosclerosis, cancer, cataracts and cell damage connected to ischaemia and reperfusion (19,20).

Clinical studies to demonstrate that vitamin E supplements can help to delay or even prevent coronary heart disease. It is also stated that vitamin E can help prevent prostate cancer (11). According to trial concerning vitamin E and cancer; twelve studies, which included 167025 participants, met the inclusion criteria. There were no statistically significant differences in total mortality [relative risk, 0.99; 95% CI, 0.96-1.03] among the different groups of patients included in this meta-analysis (11). It was found that vitamin E was associated with a significant reduction in the incidence of prostate cancer [relative risk, 0.85; 95% CI, 0.73-0.96, number needed to treat=500] (11).

Recent reports suggest that vitamin E may also display gene expression modulation activities. It is shown that treatment of cultured hepatocytes with (RRR)-alpha-tocopherol alters the expression of multiple genes and that these effects are distinct from those elicited by another antioxidant (12). It was performed that genes modulated by vitamin E include those that encode key enzymes in the cholesterol biosynthetic pathway (12). These observations indicate that vitamin E possesses novel transcriptional activities that affect fundamental biological processes.

Dietary Value of Vitamin E

1 IU of vitamin E is the activity of 1 mg of all-*rac*-*α*-tocopheryl acetate, but the activity is nowadays expressed a milligrams of *α*-tocopherol equivalents (14). For determining the total vitamin E activity of mixed diets in the USA, total mg of *α*-tocopherol equivalents was calculated. The mg of *β*-tocopherol, *γ*-tocopherol and *β*-tocopherol-3 can be multiplied by factors of 0.5, 0.1, and 0.3, respectively, and added to the mg of α-tocopherol to give the total mg of *α*-tocopherol equivalents (14).

$$\Sigma\, mg\ T.equivalents = 0.5(\beta\text{-T}) + 0.1(\gamma\text{-T}) + 0.3(\beta\text{-T-3}) + (\alpha\text{-T}) \qquad (14)$$

Especially, the best dietary sources of vitamin E are edible vegetable oils but some cereals and infant formula are the good sources of vitamin E (Table 2.2.1.1- 2.2.1.3).*α*-tocopherol level of vegetable oils is about 60 percent of the their total tocopherols. Oil processing (refining, deodorizing), boiling of vegetables, baking or storage of foods can result in substantial tocopherol losses (2).

Infant milk formulas can be considered as one of the principal sources of tocopherols (vitamin E) during this crucial step of baby life. Infant formulas contain tocopherols arising from oil combination used in manufacturing process and it may not adequate for baby nutrition. Tocopherols are added to the infant formulas either to improve the vitamin E supply or to prevent lipid oxidation. For this purpose, the use of α-T (D and DL) and *α*-tocopherol acetate (*α*-TAc) (D and DL) is permitted by law (15,23). It is reported that the mean contents of α-, γ- and δ- tocopherol were in powdered 'starting' and 'follow-on' infant formulas as 15.7-16.6; 2.4-3.9 and 0.11-1.35 mg/100 g, respectively (15,30). Tocopherols which have antiatherosclerotic, anticarcinogen, and antisterilitic effects are strong antioxidants for the body. Tocopherol level is inadequate in premature infants and hemolytic anaemia may develop if it is also deficient in breast milk and if the infant is fed with formulas containing rich levels of polyunsaturated fatty acids (PUFAs) but low levels of vitamin E. In this context, the vitamin E composition of breast milk is important (31, 32).

Transplasental delivery of vitamin E to the fetus is limited, although total body stores increase in late gestation, together with the amount of the adipose tissue. Vitamin E is higher in colostrum than mature milk, which contains approximately 3 IU/L(1 IU=1.5 mg). Thus, mature human milk comes close to meeting the DRI of 3 IU per day of vitamin E. Supplementation of the maternal diet with relatively large amounts of vitamin E is necessary to increase the milk levels of this vitamin (31).

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Table 2.2.1.1. Vitamin E contents of some fats & oils (as mg/100g)

FATS & OILS	
soybean	96-115
palm oil	89-117
corn oi	78-109
cottonseed oil	78
sesame oil	82-137
canola	65
sunflower oil	47-67
safflower	49-80
rice bran	9-160
peanut	37
cocoa butter	20
olive oil	5.1
butter	1.1-2.3
coconut	1.0-3.6

Source: Eitenmiller,1997 ; Tokuşoğlu *et.al*.,2003

Table 2.2.1.2. Vitamin E contents of and cereals & cereal products and nuts (as mg/100g)*

CEREALS	
wheat	7.0-10.0
rye	2.2-5.7
oat	1.8-4.9
rice (with hulls)	2.9
rice (polished)	0.4
corn	9.5
whole wheat meal	3.7
wheat flour	2.3-5.4
whole rye meal	2.0-4.5
white bread	2.15
crisp bread	4.0
whole rye bread	1.3
corn grits	1.17
oat flakes	3.85
corn flakes	0.43
NUTS (α-Toc.)	
almond	11.0
hazelnut	6.5
peanut	3.5
pistachio	1.0-5.24
pican	0.6
walnut	0.3

* For nuts: porsion *1 ½ ounce.*

Source: *Thaler ,1967* ; USDA (2002); Tokuşoğlu,2007

Table 2.2.1.3. Vitamin E contents of and some consumed foods (as mg/100g)

SEAFOOD	
crab,frozen	5.9
herring	1.8
mackerel	1.6
trout	1.35
MEAT	
beef liver	0.9-1.6
chicken meat	0.15-0.4
lard	0.6
VEGETAB.	
peas	4.0-6.0
cabbage	2.0-3.0
beans	1.0-4.0
tomato	0.9
spinach	0.2-6.0
lettuce	0.2-0.8
carrot	0.2
onion	0.3
EGG	
egg yolk	3.0
egg	0.5-1.5
DAIRY	
milk	0.02-0.15
infantformula	8.93-27.72
cheese	0.4

Source: Thaler,1967 ; Akalın & Tokuşoğlu, 2004

Vitamin E level in breast milk of Turkish mothers was 9.84 µg/ml whereas 7.7 µg/ml in a study in East-Jakarta and 9.7 µg/ml in a study in Germany (31,34,35). China breast milk level of Vitamin E was reported as 6.98 μg/100 ml which is considered to be low when compared with those from Europe, USA, and Japan (36).

Good nourishment of the mothers can provide balanced composition of fat-soluble vitamins containing α-tocopherol in breast milk. The total lipid of breast milk is not strongly affected by the diet, but is related to maternal body composition (31,33). It was reported that there was not any statistically significant relation between vitamin E levels in mothers' milk and frequency of consumption of any type of food (p>0.05) (31). There was not any significant relation between as body mass index (BMI) of the mothers and that vitamin levels in their milk,either (31).

Plasma tocopherol levels vary with the total plasma lipid levels. and normally, the plasma α-tocopherol level is 5 to 20 μg/mL (13).

Table 2.2.1.4. Dietary Reference Intake Values for Vitamin E by Life Stage Group

	RDA[b]			UL[c]		
Group	**mg/day**	**IU *RRR***	**IU *all*-rac**	**mg/day**	**IU *RRR***	**IU all-rac**
Adult	15	23	34	1000	1500	1100
Pregnancy	15	23	34	1000	1500	1100
Lactation	19	29	43	1000	1500	1100

*For Adult= Criterion: Intakes sufficient to prevent hydrogen peroxide-induced hemolysis *in vivo.*

*For Pregnancy= Adult EAR

*For Lactation= Adult EAR plus average amount secreted in human milk

[EAR=(Estimated Average Requirement).The intake that meets the estimated nutrient needs of half the individuals in a group].

[RDA= (Recommended Dietary Allowance). The intake that meets the nutrient needs of almost all (97%--98%) of individuals in a group].

[UL= (Tolerable Upper Intake Level). The highest level of daily nutrient intake that is likely to pose no risk of adverse health effects in almost all individuals].

***Source*:** Adapted from Food and Nutrition Board, Institute of Medicine in *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, Carotenoids,* National Academy Press, Washington, 2000, p.186-283.

According to Panel on Dietary Antioxidants and Related Compounds, FNB Institute of Medicine at 2000, the dietary reference intakes (DRIs) for vitamin C, vitamin E, selenium, carotenoids were published (37). The 2000 RDA for adults (both men and women ≥19 years) defined as 2*R*-α-tocopherol is 15 mg/day and defined as *all*-rac is 34 IU (TABLE 2.2.1.4.) (37). Increased intake of polyunsaturated fatty acids increases the need of vitamin E (2). Supplement pills containing vitamin E with natural stereochemistry (*RRR*-*α*-tocopherol) (D-*α*-T) and containing with synthetic (*all-rac*-*α*-tocopherol) (DL-*α*-T) are the most important source of vitamin E in USA diet (8).

Deficiency of Vitamin E

Dietary vitamin E deficiency is common in developing countries. Vitamin E deficiency causes degeneration of the axons of neurons (nerve cells) resulting in neurologic deficits, and fragility of red blood cells which is generally diagnosed as hemolytic anemia (13). Vitamin E deficiency may develop that genetic defects in the tocopherol transfer protein, genetic defetcs in lipoprotein synthesis or may develop as a result of fat malabsorbtion problems (8).

Retinis pigmentosa generally accompanies vitamin E deficiency in humans. It is shown that *α*-tocopherol supplementation (400 IU/day) with 15,000 IU/day of vitamin A stops or slows the retinis pigmentosa progression. Children with cystic fibrosis can become vitamin E deficient owing to the impaired secretion of pancreatic digestive enzyme causes steatorrhea and vitamin E malabsorbtion (8).

2.2.2. The Biochemistry, Functionality and Dietary Reference Intakes of Vitamin A and Carotenoids

Vitamin A:

Vitamin A-active compounds are represented by retinoids (as vitamin A) and the carotenoid precursors of vitamin A (provitamin A carotenoids) (52). Vitamin A is generic term for a group of closely related compounds also known as retinoids. In addition to its role as a chromophore in retina for vision, vitamin A is crucial micronutrient in human diet for reproduction, growth, cell metabolism, cell and tissue differentiation, haematopoiesis, bone development, pattern formation during embryogenesis and maintenance of the immune system (51,52,54,55,31).

The various retinoids (FIGURE 2.2.2.1) are present in animal tissues as a result of the enzymatic cleavage of ingested provitamin A carotenoids (52).

Dietary vitamin A is stored in the liver, normally, the liver stores 90% of the body's Vitamin A. To use Vitamin A, it is secreted into the bloodstream. The body releases it into the circulation bound to prealbumin (transthyretin) and retinol-binding protein (52,53,2).

The circulating retinol is take up by target cells and oxidized in part to retinoic acid, that induces the protein synthesis via the direct control of gene expression. This gene activation establishes vitamin A (in the form of its metabolite, retinoic acid) as a hormone, similar to the steroid hormones and the thyroid hormone (52).

Vitamin A is important for reproduction of male and female. Retinol is required for normal spermatogenesis in the male whereas it is crucial for conception and normal fetus development in the female. Retinol is required for the formation of rhodopsin, a photoreceptor pigment in the retina. It is reported that vitamin A helps maintain epithelial tissues (52,2).

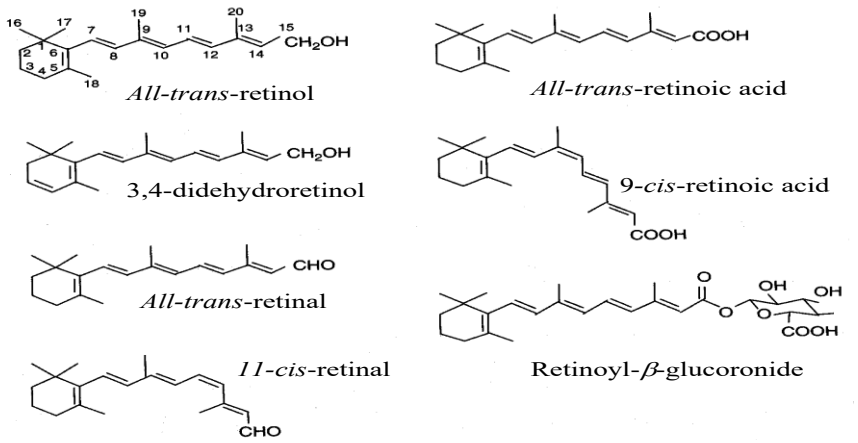

Figure 2.2.2.1. Retinoid family and major metabolites in foods. ***Source*:** Ross CA, Harrison EH. 2007. Vitamin A: Nutritional Aspects of Retinoids and Carotenoids. In *Handbook of Vitamins*. Zempleni J, Rucker RB, Mc Cormick DB, Suttie JW.4th edition. p:1-39. CRC Press , Taylor & Francis Group, Boca Raton, FL

Retinol structure comprises a cyclohexenyl (*β*-ionone) ring attached at the carbon-6 (C-6) position to a polyene side chain.whose four double bounds give rise to *cis-trans* (geometric) isomerism as shown in Figure 2.2.2.1. (51,52).

The *all-trans* form is the most active biologically and more stable than the other retinoids (53). Retinol (Figure 2.2.2.1) is most often present in tissues in esterified form, where the fatty acyl group is usually palmitate with lesser amounts of stearate and oleate esters. Esterification protects the hydroxyl group from oxidation. Retinyl esters are the major form of vitamin A in the body as a whole and the predominant form (more than 95%) in chylomicrons, cellular lipid droplets, and milk fat globules (52). The retinoids elicit the functions of vitamin A through their ability to regulate gene expression at specific target sites in the nucleus of cells (54,55). *All trans*-retinoic acid (Figure 2.2.2.1) is the most bioactive form of vitamin A. Retinoic acid is also the most potent natural ligand of the retinoid receptors, RAR and RXR. 9-*cis*-retinoic acid is capable of binding to the nuclear receptors may be a principal ligand of the RXR. 13-*cis*-retinoic acid is present in plasma, often at a level similar to *all-trans*-retinoic acid, and its therapeutic effects are well reported (51).

Carotenoids:

Carotenoids are yellow, orange, red, or violet pigments that are responsible for the color of many fruit and vegetables (56). Certain almost-colorless carotenoids also exist, such as phytofluene,that fluoresces intencely under ultraviolet (UV) irradiation. Carotenoids are synthesized by photosynthetic plants and some algae and bacteria, but not by animal tissues (Figure 2.2.2.2.).

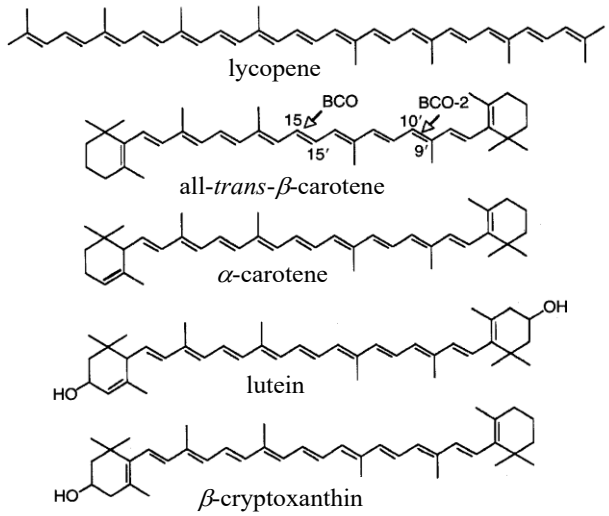

Figure 2.2.2.2. Major carotenoids in foods ***Source***: Ross CA, Harrison EH. 2007. Vitamin A: Nutritional Aspects of Retinoids and Carotenoids. In *Handbook of Vitamins*. Zempleni J, Rucker RB, Mc Cormick DB, Suttie JW.4th edition. p:1-39.

Carotenoids are classified as provitamins and can be considered chemically as derivatives of $C_{40}H_{56}$ polyene composed of eight isoprenoid units (Figure 2.2.2.2.). Derivatives are formed by a variety of reactions that include cyclization, hydrogenation, dehydrogenation and insertion of oxygen. In this context, carotenoids are classified as four groups as shown below (57).

- ❋ Carotenoid Hydrocarbons
- ❋ Carotenoid Alcohols(Xanthophylls)
- ❋ Carotenoid Ketons
- ❋ Carotenoid Acids

Hydrocarbon carotenoids are known as "*carotenes*", and the oxygenated derivatives are termed "*xanthophylls*" (52,57)

carotene and other provitamin carotenoids, contained in green leafy and yellow vegetables and deep-or bright-colored fruits, are converted to Vitamin A. More than 600 carotenoids have been identified, but most nutrition research has focused on the several carotenoids: *α*-carotene, *β*-carotene, lycopene, lutein, zeaxanthine, *β*-cryptoxantine, *β*-apo-8'-carotenal, *β*-apo-8'-carotenoic-acid and canthaxantine (57). Carotenoids are absorbed better from vegetables when they are cooked or homogenized and served with some fats or oils (58).

Beta-carotene occurs widely in plant products and has a high vitamin A activity. One molecule of *β*-carotene, could yield two molecules of vitamin A (FIGURE 2.2.2.2.). After cleavaging of *β*-carotene, the first reaction product is retinal, that is reduced to retinol. To have vitamin A activity, the carotenoid molecule must incorporate a molecule of retinol,i.e.,an unsubstituted *β*-ionone ring with an 11-C polyene chain. *β*-carotene ($C_{40}H_{56}$), the most ubiquitous provitamin A carotenoid, is composed of two molecules of retinol joined tail to

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tail; therefore the compound has maximal (100%) vitamin A activity The chemical structures of all other provitamin A carotenoids incorporate one molecule of retinol and thus theoretically contribute 50% of the biological acitivity of β-carotene (2,51).

Lycopen (FIGURE 2.2.2.2.) is an acyclic carotenoid with 11 linearly arranged conjugated double bonds, which is found only in few foods. Tomatoes and tomato products are the main foodstuffs contributing the dietary intake of lycopene. In addition, apricots, guavas, watermelons, papayas, pink grapefruits, rosehips contain lycopene (59-61). Due to the lacking the β-ionone ring structure, lycopene does not have any provitamin A activity. Lycopene is an efficient antioxidant and singlet oxygen quencher. Recent epidemiological researches reported that lycopene has preventive agent for cardiovascular diseases and cancer (59-61).

Lutein (Figure 8.2.2.3.) and its stereo isomer zeaxanthine (Figure 2.2.2.4.) are members of the xanthophyll family of carotenoids. The xanthophylls lutein and zeaxanthin have attracted a lot of interest due to the it was presumed that both compounds may prevent adult macula degeneration (AMD),age-related cataract information (38-41) and may delay chronic disease (39-40) and some forms of cancer (42).

As antioxidants, lutein and zeaxanthin can also inhibit the formation of damaging free radicals by quenching singlet oxygen Conjugated double bonds are highly effective in quenching singlet oxygen; therefore, the fact that zeaxanthin has an extra conjugated double bond may take it a better antioxidant than lutein (43,50) (Figure 2.2.2.5).

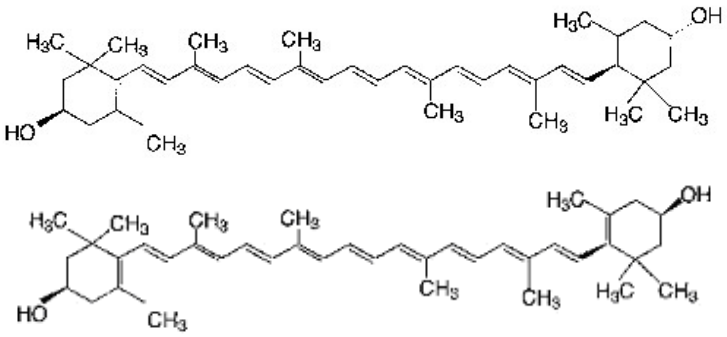

Figure 2.2.2.4. Zeaxanthine

Morever dark-green leafy vegetables (especially spinach, kale, collard, broccoli, mustard), corn, orange peppers, orange, kiwi, tangerine and various kinds of squash (44,45); egg yolks also serve as traditional source of xanthophylls (46,47). Especially lutein and zeaxanthin in yolks might be highly bioavailable because of their association with the lipid matrix of the egg yolk (46,50) (Figure 2.2.2.5)

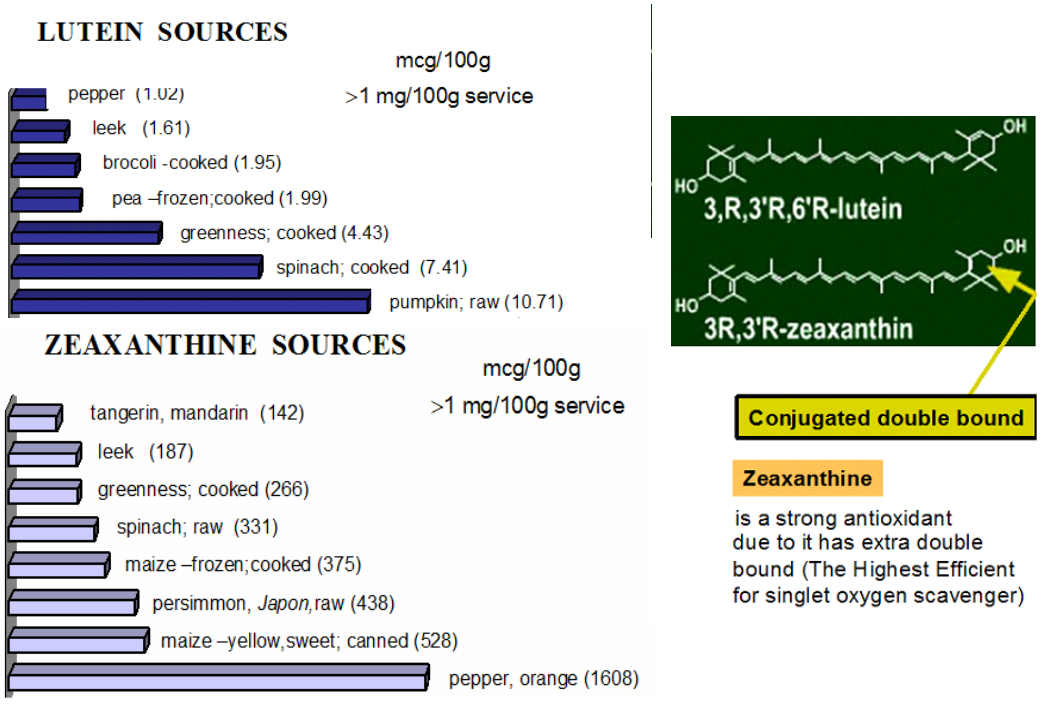

Figure 2.2.2.5. Lutein and zeaxanthine levels in foods (57,62)

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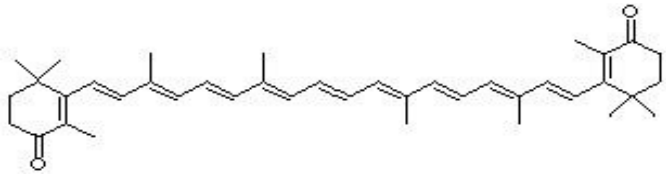

Figure 8.2.2.6. Canthaxanthine

It has been observed that canthaxanthin (Figure 2.2.2.6.) has antioxidant capabilities *in vitro*. It is showed that canthaxanthin was able to reduce UV-light induced tumours (48,49).

In medium egg yolks that have 61.04 g of average total weight and 17.84 g of yolk weight; lutein as 844.57 μg/yolk (avg.), zexanthine, as 12.95 μg/yolk (avg.) and canthaxanthine as 11.4 μg/yolk (avg.) were determined, based on dry weight (p<0.01) (50). It has been put forwarded that a standardization should be necessary regarding in enhanced functional egg (enhanced nutraceutical egg) with xanthopyll carotenoids (50). In the same research, afterwards the heating process, it is also reported that lutein decreased as 21.43% in soft-boiled yolk and as 38.22% in hard-cooking yolk whereas zexanthine reduced as 1.88% in soft-boiled, as 32.53% in hard-cooking yolk and canthaxanthine decreased as 6.99% in soft-boiled yolk and as 45.80% (p<0.01) (50).

Dietary Value of Vitamin A

The vitamin A value of foods has traditionally been expressed in International units (IU). One IU is defined as the amount of vitamin A activity contained in 0.334 μg of *all-trans*-retinyl acetate, that is equivalent to 0.300 μg of *all-trans*-retinol (52).

$$\text{RE} = \mu\text{g retinol} + \frac{\mu\text{g } \beta\text{-carotene}}{6} + \frac{\mu\text{g other provitamins}}{12}$$

It is reported that the IU values can be corverted to retinol equivalents.The convert IU into RE on the basis of retinol, 1RE= 1μg retinol and 1 IU= 0.3 μg retinol. Therefore; 1 RE= 1/0.3= 3.33 IU vitamin A activity from retinol (52). In the RE system, 1 μg retinol= 6 μg β-carotene, whereas in the IU system, 1 μg retinol= 2 μg β-carotene. It is reported that, the convertion of IU to RE on the basis of β-carotene, one must first multiply the IU by a factor of 3 (6/2), to make the equivalency the same as that of the RE system, and then multiply by 3.33 (52).

In 2001, the U.S. Institute of Medicine recommended the replacing the RE with the retinol activity equivalent (RAE) and the redefining the average equivalency values for carotenoids in foods in comparison with retinol (53). 1 μg RAE is defined as 1 μg of *all-trans*-retinol, and thus is the same as 1 μg RE. Both are equal to 3.3 IU of retinol. The equivalency of provitamin A carotenoids and retinol in the RAE system as shown below. RAE terminolgy is not yet fully utilized, the vitamin A values in some food tables, food labels, and supplements are still expressed in RE or IU.

Figure 2.2.2.7. shows digestion and absorption mechanism of antioxidants.

This describes the path of a general antioxidant compound from the food matrix through the human body (41). For a food component to be absorbed, it must be released from the matrix and be accessible to the brush-border of the small-intestine in a form that it can be absorbed by the enterocyte either by passive diffusion or by an active transporting system (41). That process is time-dependent. It depends upon an array of variables containing: (I) the state of food (raw, cooked and/or processed), (II) the presence and efficicency of digestive enzymes and other endogenous digestants, (II) the composition of meal (41). For carotenoids, food processing is important facilitator for carotenoid bio-accessibility. The availability for absorption is enhanced by, for instance, the carotenoid transfer to the lipid phase during cooking in the presence of oil, and by disruption of the cellular matrix during mastication (41). Vitamin E, carotenoids and other lipid soluble components are absorbed via the mucosa of small intestine, mainly in duodenum (63,64). The carotenoids are transported through the enterocyte from the lumenal to the serosal side. These carotenoids are incorporated into lipoproteins, that are then released into the systemic circulation (41, 65) (FIGURE 2.2.2.7.).

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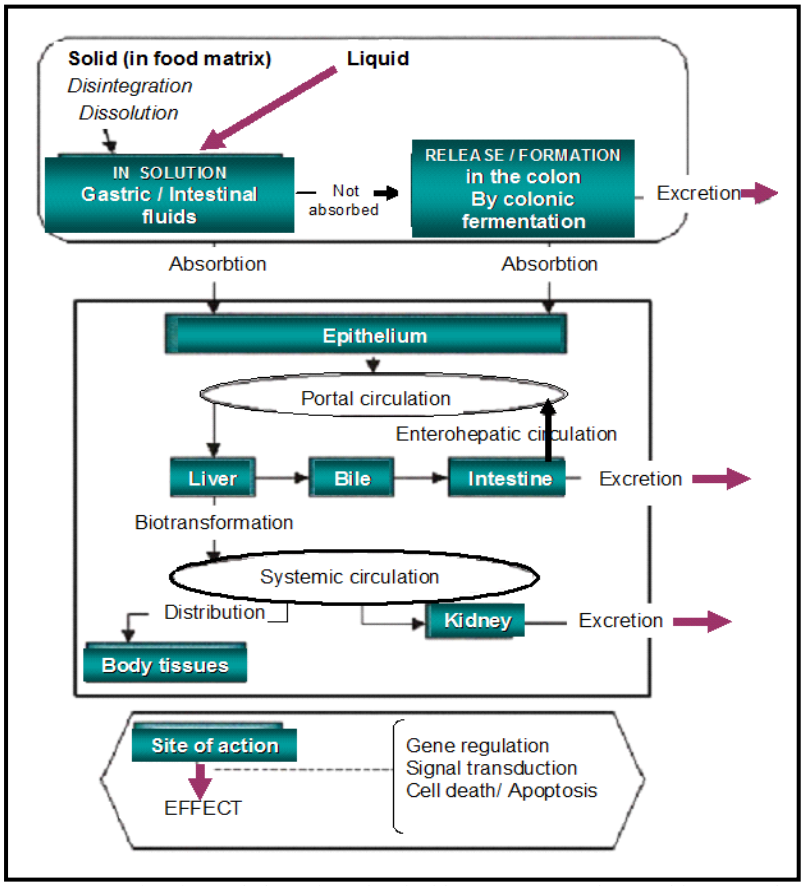

Figure 2.2.2.7. Digestion and absorption of antioxidants. ***Source*:** Alves-Rodrigues A, Shao Andrew 2004. The Science Behind Lutein. *Toxicology Letters*. 150, 57-83.

Table 2.2.2.1. Vitamin A and carotene levels of some foods**Source: de Man,1999* ; USDA,2004; Demirci,2003

FOOD	Vitamin A (IU/100g)	Carotene (mg/100g)
Animal Sources		
Beef liver	6000-14000	-
Calf's liver (4 oz-wt)	30485	-
Butter (May-Nov.)	2363-3452	0.43-0.77
Cheddar cheese	553-1078	0.07-0.71
Milk	110-307	0.01-0.06
Egg yolk (raw)	1000-4000	
Egg (boiled)	165-488	0.01-0.15
Herring (canned)	178	0.07
Sole	450	
Beef (grilled sirloin)	37	0.04
Vegetables & Fruits	**(1 cup)**	
Carrots, raw	34317	-
Spinach (boiled)	14742	6.0
Sweet potato, baked, with skin	13107	-
Turnip greens (cooked)	7917	-
Kale (boiled)	9620	-
Cantaloupe, cubes	5158	-
Winter squash, baked, cubes	7292	-
Mustard greens (boiled)	4243	-
Collard greens (boiled)		-
Bell peppers (red, raw, slices)	5244	-
Tomato (ripe)	1121	-
Tomato (canned)	-	0.5
Asparagus (boiled)	970	-
Apricot (1 each)	914	-
Papaya(1 each)	863	-
Broccoli (steamed)	2281	2.5
Peach	-	0.34
Cabbage (shredded,boiled)	198	0.3

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Table 2.2.2.1.shows vitamin A and carotene content of some foods whereas TABLE 2.2.2.2. indicate vitamin A levels of some fortified foods.

Table 2.2.2.2. Vitamin A levels of some fortified foods*

Fortified Foods	Vitamin A (mg/100g)
Margarines	~0.8
Powdered breakfast drinks	3-6
Ready-to- eat cereals	0.7-1.5

Calf liver is an excellent source of preformed vitamin A. Cow's milk and eggs are good sources of preformed vitamin A. NHANES data 2001-2002 for food consumption in the USA showed that the major contributors to the intake of preformed vitamin A are milk, margarine, eggs, beef, liver,ready-to-eat cereals, whereas the excellent sources of provitamin A carotenoids are carrots, sweet potatoes, cantaloupes and spinach (69).

As a vitamin supplement, vitamin A is available as retinol and retinyl-palmitate. Retinoic acid is the form of vitamin A found in medications prescribed for the treatment of skin disorders (68).

Table 2.2.2.3. shows the dietary reference intake values for vitamin A by life stage group according to the Institute of Medicine at 2002 (53). The dietary reference intakes (DRIs) established by the Institute of Medicine (IOM) are a set of reference values including the estimated average requirement (EAR), recommended dietary allowance (RDA), adequate intake (AI), and tolerable upper intake level (UL).

During pregnancy, the intake and hepatic reserves of maternal vitamin A are essential to guarantee the transference of this micronutrient to the foetus, and its first source of the nutrient. The observed average vitamin A intake of women in developing countries (660 μg retinol equivalents/day) is less than one-half of the amount taken in developed countries (1,540 μg retinol equivalents/day) (54,55). Adequate amounts of vitamin A cross the placenta to the foetus, especially in the last trimester; however, vitamin A stores in the newborn liver are relatively low compared with those of older infants and children (54,55).

Table 2.2.2.3. Dietary Reference Intake Values for Vitamin A by Life Stage Group

Group	RDA (μg/day)[a]	UL (μg/day)[b]
■ Infants		
0-12 months	400[c]	600
■ Children		
1-3 years	300	600
4-8 years	400	900
■ Adolescent and adult males		
9-13 years	600	1700
14-18 years	900	2800
19 to ≥70 years	900	3000
■ Adolescent and adult females		
9-13 years	600	1700
14-18 years	700	2800
19 to ≥70 years	700	3000
■ Pregnancy		
<18 years	750	2800
19-50 years	770	3000
■ Lactation		
<18 years	1200	2800
19-50 years	1300	3000

[a]As retinol activity equivalents (RAEs)
[b]As µg preformed vitamin A (retinol)
[c]Adequate intake (RAEs)
Source: Food and Nutrition Board, Institute of Medicine in *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc,* National Academy Press, Washington, 2002, p.8-9.

Human breast milk is an excellent source of vitamin A. The vitamin A content of human milk is more influenced by maternal dietary intake than by mother's vitamin A status. The sources of vitamin A for milk synthesis are plasma retinol-binding protein (RBP)-retinol and retinyl esters in chylomicrons. The former source is relatively constant after the initial decrease regardless of vitamin A status (liver stores), where the latter is directly related to the maternal intake (54,55)*.

Table 2.2.2.4. Retinol concentration in breast milk and plasma.

Retinol concentration*	Total milk (*n*=46)	İzmir group (*n*=26)	Manisa group (*n*=20)
	Plasma (μmol/l)		
Average	126.0 ± 36.0	123.0 ± 17.0	129.0 ± 23.0
Minimum	70.0 ± 12.56	70.0 ± 12.56	74.0 ± 13.64
Maximum	212.0 ± 30.7	184.0 ± 21.5	212.0 ± 30.7
	Milk (μg/100ml)		
Average	82.47 ± 17.0	81.0 ± 10.1	84.0 ± 9.02
Minimum	56.83 ± 8.84	57.92 ± 6.92	56.83± 8.84
Maximum	113.8 ± 23.2	112.83± 16.76	113.8± 23.2

*(p<0.05)

***Source*:** Adapted from by Tokuşoğlu *et.al.* **2008.** *Int.J.Food Sci.Nutr*.59(2), 166-174 Retinol and alpha-tocopherol concentrations in breast milk of Turkish lactating mothers under various socio-economic status

Table 2.2.2.4. shows that retinol concentration in breast milk and plasma of lactating mothers living in two different area of Turkey (31). If the plasma retinol concentration is less than 20.05 μg/100 ml over 15% of a specific population, vitamin A deficiency is defined to be of public health importance. It was reported that none of the mothers in whom plasma vitamin A levels were found had a value less than 20.5 μg/100 ml and vitamin A concentrations of both plasma and breast milk were appropriate scale for investigated lactating womans in above-mentioned study (Table 2.2.2.4.) (31).

A retinol level in breast milk less than 30.09 μg/100 ml is considered to be consistent with vitamin A deficiency in lactating women. Prevalance rates of <10%, ≥10% to <25%, and ≥25% of breast milk retinol <30.09 μg/100ml are considered to indicate mild, moderate, and severe vitamin A deficiency, respectively, as a public health problem based on WHO (70).

Dietary deficiency of vitamin A is quite common in developing countries, and is associated with the high incidence of blindness, viral infections and child mortality which occurs in impoverished populations (53). Vitamin A deficiency is a primary cause of xerophthalmia, that is manifested as night blindness and corneal abnormalities leading to irreversible blindness. Enough vitamin A level is absorbed and stored quickly, and released and used slowly, to prevent the development of xerophthalmia over an extended period (8,71).

It is estimated that vitamin A administered at doses of 200,000 IU (60 mg retinol) every 6 months would reduce total mortality by 35% in preschool children (72).

Vitamin A reduced measles-related morbidity and mortality (8, 73). Owing to the protective role of vitamin A, WHO/UNICEF recommended in 1987 that in those countries where the measles fatality rate is 1% or greater, all the children diagnosed with measles should receice 30-60 mg of vitamin A immediately (8,74). The American Academy of Pediatrics has also recommended vitamin A in the treatment of high-risk children with measles (75).

Vitamin A deficiency also affects the skin health, the health of hair, eyes, and immune system, though loss of appetite, bone abnormalities. A tell-tale sign of vitamin A deficiency is hyperkeratosis, a goose bump-like appearance of the skin caused by excessive production of keratin (a protein found in skin) that blocks hair follicles. Growth retardation are also associated with inadequate intake of vitamin A (53).

Vitamin A can cause side effects when taken in excessive amounts. High storage amounts of vitamin A in the body (hypervitaminosis A) that can lead to toxic semptoms. Most causes of vitamin A toxicity are due to accidental ingestion of doses exceeding 660,000 IU (200 mg of retinol equivalents) and 330,000 IU (100 mg of retinol equivalents) by adults and children, respectively (53). Signs of vitamin A toxicity include nausea, loss of appetite, vomiting, headache, irritability, dizziness, blurred vision, lack of muscular coordination, abnormal liver functions, pain in weight-bearing bones.

2.2.3. The Biochemistry, Functionality and Dietary Reference Intakes of Vitamin D

Vitamin D is represented by cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2), that are structurally similar secosteroids derived from th UV irradiation. The generic term vitamin D designates a group of chemically related compounds which possess antirachitic activity. Members of D-family are derived from the cyclopentanoperhydrophenantrene ring system as shown in FIGURE 2.2.3.1. (52,112).

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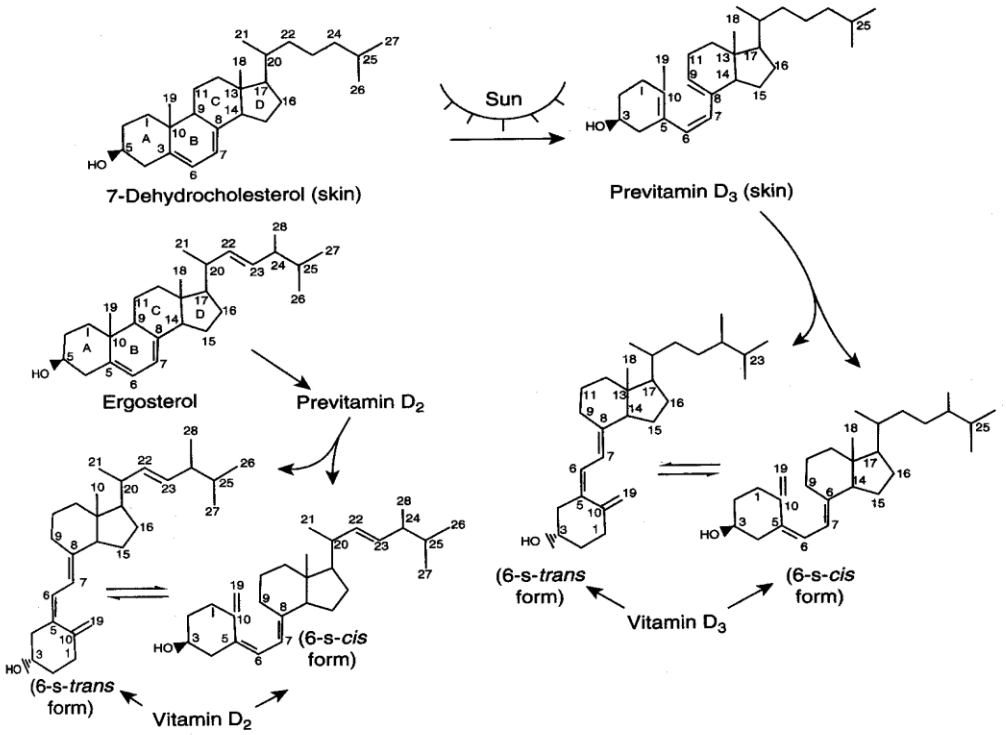

Figure 2.2.3.1. The chemistry and irradiation pathway for production of vitamin D_3 and vitamin D_2.

It is shown that provitamins D were converted to vitamin D on treatment with UV radiation (Figure 2.2.3.1). The primary structural requirement for a provitamin D is a sterol with C-5 to C-7 diene system in ring B (112). It is determined that the conjugated double-bond system is a chromaphore, which on UV irradiation initiates a series of transformations resulting in the production of the vitamin D secosteroid structure (Figure 2.2.3.1) (112).

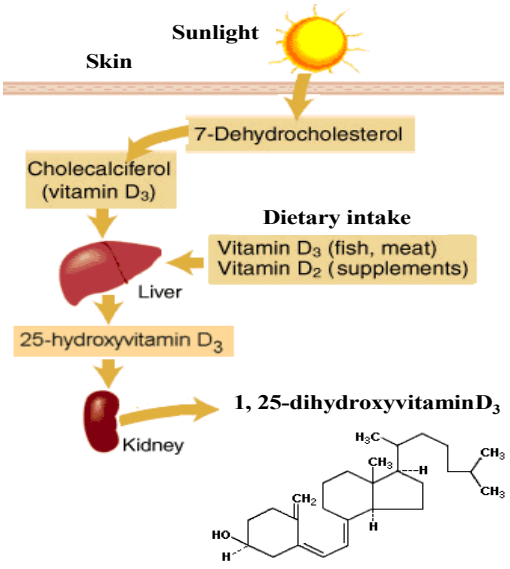

Figure 2.2.3.2. The metabolic pathway for production of 1,25-dihydroxyvitamin D_3 from kidney.

Vitamin D_3 that is described as 1,25-dihydroxyvitamin D_3 is synthesized in skin by exposure to sunlight (ultraviolet radiation). Vitamin D3 is metabolized by the liver to 25(OH)D, which is then converted by the kidneys to 1,25(OH)2D (1,25-dihydroxycholecalciferol, calcitriol, or active vitamin D hormone). 25(OH)D, the major circulating form, has some metabolic activity, but 1,25(OH)2D is the most metabolically active (FIGURE 2.2.3.2).

Vitamin D intake has been hypothesized to reduce the risk of several types of cancer. Vitamin D and its analogues have demonstrated anticancer activity *in vitro* and in animal models (76).

However, the risk of colorectal cancer in relation to dietary vitamin D remains controversial (76). Studies that combine multiple sources of vitamin D or examine serum 25(OH)D_3 usually find that above-average vitamin D intake and serum metabolite concentrations are associated with significantly reduced incidence of colorectal cancer (76).

1,25-dihydroxyvitamin D_3 obtained in the diet chiefly in fish liver oils and egg yolks. In some developed countries, milk and other foods are fortified with vitamin D.

In vitro and animal studies indicate that vitamin D may have anti-cancer benefits, including against progression and metastasis, against a wide spectrum of cancers (77). It was reported that supporting an anti-cancer effect of vitamin D is the ability of many cells to convert 25(OH)D, the primary circulating form of vitamin D, into 1,25(OH)2D, the most active form of this vitamin (77). It was declared that higher 25(OH)D levels through increased sunlight exposure or dietary or supplement intake inhibit colorectal carcinogenesis is substantial. The suppression of circulating 1,25(OH)2D levels by calcium intake could explain why higher calcium and milk intakes appear to increase risk of advanced prostate cancer (77).

The association of specific circulating 25(OH)D concentrations with bone health outcomes in children, women of reproductive age, postmenopausal women and elderly men; the effect of dietary intakes (foods fortified with vitamin D and/or vitamin D supplementation) and sun exposure on serum 25(OH)D; the effect of vitamin D on bone mineral density (BMD) and fracture or fall risk; and the identification of potential harms of vitamin D current reference intakes (78). It was reported that intakes from vitamin D-fortified foods (11 randomized controlled trials RCTs) consistently increased serum 25(OH)D in both young and older adults. It is also shown that vitamin D_3 in combination with calcium results in small increases in BMD compared to placebo in older adults although quantitative synthesis was limited due to variable treatment durations and BMD sites (78).

In humans, patients with hypocalcemic vitamin D-resistant rickets type II have high circulating vitamin D levels and vitamin D resistance due to expression of a dysfunctional vitamin D receptor (VDR) (79).

One of the major functions of vitamin D is maintaining proper calcium and phosphorous levels in the blood. The simply description of the most metabolically active form of vitamin D (1,25-dihydroxy-cholecalciferol) is “Calcitriol” (80). Calcitriol works with parathyroid hormone (PTH) to maintain proper levels of calcium in the blood. Low levels of calcium in the blood stimulate the secretion of PTH from the parathyroid gland. when blood levels of calcium are too high, calcitriol decreases the intestinal absorption of calcium and stimulates the bones to take up calcium, thereby decreasing blood calcium levels. Without adequate phosphorus, bone cannot be properly mineralized, which contributes to the defects seen in osteomalacia. In addition, the new bone cells being laid down by the osteoblasts (the cells that create new bone) absorb more water and swell, causing the bone pain associated with osteomalacia (80).

Vitamin D may play an important role in regulating cellular growth and function in human brain cells. In mice studies, vitamin D has been found to have a significant effect on brain cell (neuronal) growth and division (80). Vitamin D also helps regulate immune system activity, preventing an excessive or prolonged inflammatory response. Immune cells of humans, specifically active T-cells, have receptors for vitamin D. For example, in multiple sclerosis, T helper cells drive the progression of the disease by recruiting other inflammatory immune cells (macrophages and inflammatory cytokines)-particularly when vitamin D is deficient. When vitamin D levels are adequate, the body switches on a vitamin-D mediated system that can help shut down the inflammation. Vitamin D's anti-inflammatory effects are seen across a wide spectrum of health conditions including hypertension, type 1 diabetes, and psoriasis (80).

One possible mechanism involves the relationship between vitamin D and renin levels, since renin, an enzyme secreted by the kidney, is important in regulation of blood pressure and heart health (80). Vitamin D deficiency accelerates progression to type 1 diabetes in diabetic-prone mice, but if they are given Vitamin D3, their risk of progression decreases by 80%. Whether this relationship holds true for humans has yet to be determined (80).

Table 2.2.3.1. shows vitamin D levels of some foods (Table 2.2.3.1.). As shown the data from Table 2.2.3.1., vitamin D that occurs naturally in unfortified foods is generally derived from animal products. Salt-water fish such as herring, salmon, sardines contain substantial amounts of vitamin D and cod fish-liver oils are extremely rich sources. (81,112). Eggs (egg yolk), beef, unfortified milk, butter supply only small amounts of vitamin D. Another food sources such as fruits and vegetables have no contain vitamin D. Therefore dietary requirements for vitamin D can only met by the fortification of suitable foods, containing margarine, butter, fresh and evaporated milk, cereals and chocolate mix (112).

Table 2.2.3.1. Vitamin d levels of some foods*

FOOD	Vitamin D (μg/1000g)	Vitamin D (IU)
Animal Sources		
Liver (beef,pork)	2-5	-
Liver, beef,cooked, $3^{1/2}$ ounces	-	15
Eggs	44	-
Eggs (1 whole)■	-	20
Fats & Dairy		
Margarine, fortified 1 tablespoon		60
Butter	2-40	-
Milk	0.9	-
Cow's milk, 2% (1 cup)	-	98
Cheese	12-47	-
Cheese (Swiss, 1 ounce)	-	12
Pudding (with vit.D fortified milk)	-	50
Seafood		
Herring oil	2,500	-
Cod liver oil, 1 tablespoon	-	1360
Salmon (chinook,baked/broiled) *	-	411
Mackerel, cooked $3^{1/2}$ ounces	-	345
Salmon, cooked $3^{1/2}$ ounces	-	360
Sardines (canned,in oil) $1^{3/4}$ounces	-	250
Tuna fish (canned,in oil) 3 ounces	-	200
Shrimp (steamed/boiled) *	-	162
Cod (baked/broiled) *	-	64

*4 oz-wt; ■vit.D. is found in egg yolk.
Source: de Man,1999 ; USDA,2004;2003

Dietary Value of Vitamin D

One IU of vitamin D is the activity of 0.025 μg of crystalline cholecalciferol. An expert committee in 1970 recommended that the intake of vitamin D be expressed as μg of cholecalciferol rather than as IU (52).

The World Health Organization has defined the International unit (IU) of vitamin D_3 as "the vitamin D activity of 0.025 μg of the international standard preparation of crystalline vitamin D_3.

By USA Food and Nutrition Board of the Institute of Medicine in current adequate intake allowance of vitamin D is 200 IU/day (5 μg/day) for all life stage group (82).

As shown in Table 2.2.3.2., for males and females ages 51-70 or more than 70, the adequate indicated level is set at 400 IU/day (10 μg/day) or 600 IU/day (15 μg/day) (82).

Table 2.2.3.2. Dietary Reference Intake Values for Vitamin D_3 by Life Stage Group

Group	RDA (IU/day)	μg/day
■ Infants	200	5
■ Children	200	5
■ Adult males	200	5
■ Adult females (18-51 years)		
Pregnancy	200	5
Lactation	200	5
■ Adult males (51 to ≥70 years)	400 (600)	10 (15)
■ Adult females (51 to ≥70 years)	400 (600)	10 (15)

[b]As μg vitamin D_3.,
Source: FNB (1997)

It is demonstrated that the influence of season and latitude on the cutaneous photochemical synthesis of vitamin D_3 (83). The maximum vitamin D_3 production occurs in summer months and the production of D_3 photochemically through exposure to sunlight is significantly higher at latitudes close to the equator and falls off significantly at higher latitudes. The little or no vitamin D_3 may be generated in winter months (83,84). It is reported that there is a serious nutritional vitamin D_3 problem for Finland (e.g.,Helsinki), Alaska (e.g.,Fairbanks) or Canada (e.g.,Edmonton) peoples due to the latitudes higher than 50° with clear atmospheric conditions. No cutaneous production of this vitamin occurs during some periods of year (85).

Dietary deficiency of vitamin D results in insufficient intestinal absorbtion and renal reabsorbtion of calcium and phosphate. The serum calcium and phosphate levels decrease whereas serum alkaline phosphatase

activity increases. Qwing to the low serum calcium levels (5-7 mg/100 ml), hyperparathyroidism occurs (86). When paratyhroid hormone (PTH) increases, along with vitamin D_3 deficiency, the bone demineralization develop. This situation result in rickets in children and osteomalacia in adults (86).

2.2.4. Utilization Strategies of Fortified Vitamin A,D,E

The level of fat soluble vitamins as A,D,E in fortified vegetable oils and margarine depends on consumption patterns and nutritional requirements. Clearly, prior to determining the level of nutrient to add to oils and fats, the use of and consumption patterns for margarine by different socioeconomic and age groups need to be determined in order to ensure that the maximum safe level of intake for each nutrient is not exceeded.Vegetable oils are suitable as vehicles for vitamins A,D,E fortification, as the production and refining of the oils is a centralized process. Vegetable oils are consumed by almost everyone; thus it is possible to improve people's access to fat soluble vitamins through fortification. Vitamin A,D,E are fat soluble, they can be uniformly distributed in oils. The stability of vitamin A is greater in oils than in any other foods and oil facilitates the absorbtion of vitamin A by the body.

Vitamin E, as tocopherol acetate, is added to fats and oils including margarine and fat spreads and breakfast cereals. The intake of vitamin E is known to be related to the total dietary intake of fat and it has been shown to enhance absorption and bio-conversion of dietary carotenoids to vitamin A. Emerging evidence indicates that daily intake of vitamin E greater than 30 IU may afford protection against the development of degenerative diseases. Optimal levels of fortification of vitamin E should be reassessed based on such scientific evidence. It is shown that fortifying margarine with vitamins A, D and E does not alter its flavor, making it an excellent carrier for these micronutrients. Foods made with fortified soybean oil including mayonnaise, fried beans, cooked rice, fried potatoes, soup, wheat tortillas, and fried meat showed excellent acceptability among consumers, who were not able to distinguish between products prepared with vitamin A fortified and unfortified oils. Studies in humans have shown that the bioavailability of vitamin A in cooked foods made with fortified soybean oil, is good.

Many important advantages exist for the edible oil as a medium for vitamin E fortification:Vitamin E completely soluble in oil and is uniformly distributed, vegetable oils consumed by all populations, absorption and utilization of vitamin E are better when consumed with fats and oils,fortification technology is simple and available, cost of fortification is relatively low and affordable for all income groups. Cost of fortification includes capital costs, such as blending equipment (tanks, propellers or agitators) and recurrent costs containing those for the premix, personnel, monitoring and evaluation. The production and fortification of margarine is carried out in a batch or continuous process. To ensure that the vitamins are uniformly distributed, mixing takes place in vertical tanks that contain turbines or propeller agitators. The vitamin A and D_3 blend mixed with warm oil in a ratio of 1:5 until a uniform solution is obtained. This premix is then incorporated into the margarine before the emulsifying process. β-karoten is also added to margarine (15-20 g/ton) of a 30% oily suspension) before the emulsifying step to enhance to color as well as to contribute to the vit A content the product.(FIG.2.2.4.1).

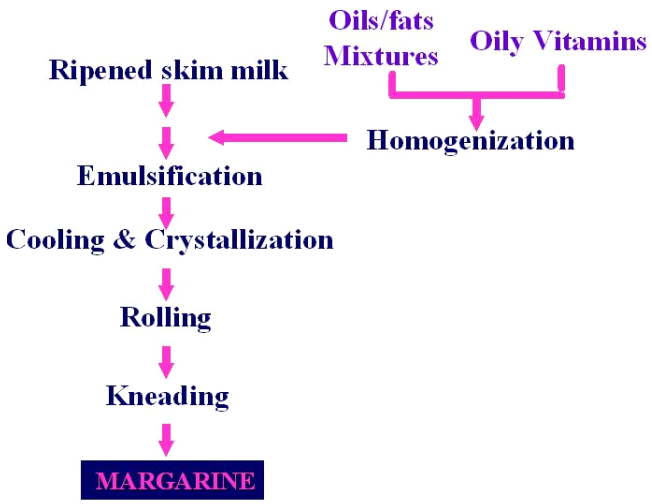

Figure 2.2.4.1. Flow chart of fortified margarine manufacturing. (source: adopted from tokuşoğlu, 2006. *Fortification Criteria of Vegetable Oils and Margarines with Alpha-Tocopherol and Tocopheryl Acetate: The Regulations on Vitamin E Labeling*. Lecture. In World Conference and Exhibition on Oilseed and Vegetable Oil Utilization, Processing, By-Products, Biodiesel, Specialty and Functional Oils, and New Applications & Technologies 14-16 August 2006, Istanbul)

 http://dx.doi.org/10.20863/nsd.37516

2.2.5. The Biochemistry, Functionality and Dietary Reference Intakes of Folic Acid

Folic acid consist of a large group of related compounds having the same basic chemical structure as folacin, or pteroylglutamic acid (PteGlu), as differing in the state of reduction and the number of glutamate residues (FIGURE 2.2.5.1) (2,87,88).

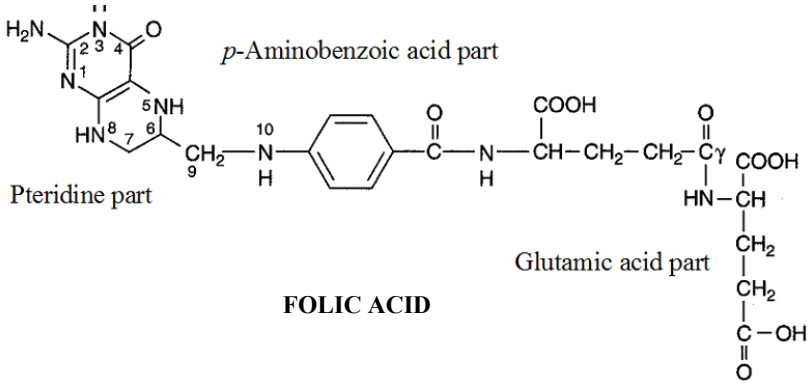

Figure 2.2.5.1. Folic Acid Basic Structure (88). ***Source*:** Adopted from Bailey LB. **2007.** Folic Acid. In *Handbook of Vitamins*. Zempleni J, Rucker RB, Mc Cormick DB, Suttie JW.4th ed. p:153-174.CRC Press , Taylor & Francis Group, Boca Raton, FL.

Folate molecule, tetrahydrofolate (THF), also called vitamin B_9, is derived from 5,6,7,8-tetrahydropteroylglutamate,that consist of a 2-amino-4-hydroxy-pteridine (pterin) moiety linked via a methylene group at the C-6 position to a *p*-aminobenzoylglutamic acid (*p*ABG) (88) (FIGURE 2.2.5.1.).

Specific one carbon (C) units which can be added at N-5 or N-10 or both N-5 and N-10 positions of the polyglutamyl form of the THF molecule include methyl (CH_3), methylene (-CH_2-), methyenyl (-CH=), formyl (-CH=O), formimino (-CH=NH) groups The pyrazine ring in THF is fully reduced at the 5,6,7,8 positions and the reduction at 7 and 8 positions yields dihydro folate (88).

Through the addition of glutamate residues by γ-peptide linkage, glutamic acid in the monoglutamate form of this vitamin can be converted to a glutamate chain (88).

Folic acid is involved in maturation of red blood cells and folacin deficiency produces a megaloblastic anemia. From 1940s to now, the therapeutic use of folic acid for the treatment of megaloblastic anemia is continuing. Since the Early 1990s, the links between folate intake and birth outcome or chronic disease risk were explored (88,94).

Biochemically, folic acid functions *in vivo* as coenzymes and carriers of one-carbon units for a number of enzyme reactions, containing aminoacid synthesis, protein and nucleic acid synthesis which are required for development of the fetal nervous system. Folic acid participates in both anabolic and catabolic reactions (89,94).

It has been recently implicated that folic acid has crucial effects in a number of non-vitamin functions including roles in various types of cancer, coronary heart diseases, the prevention of birth defects such as neural tube abnormalities which are known neural tube defects (NTDs) (89-93). Adequate folic acid intake prior to conception and throughout the first trimester of pregnancy helps prevent certain brain and spinal cord defects such as spina bifida (94).

Folic acid is more readily available than the naturally occurring food folates but may be less available from fortified foods than in aqueous solution or tablet form. Food folates have been reported to be 30-80% as available as folic acid. Folic acid is absorbed in the duodenum and upper jejunum (87,89,95).

The bioavailability of folate may be defined as the portion of nutrient and influenced by various factors containing but not limited to the following: (a) chemical form of folate, (b) food matrix, (c) the chemical environment in the intestinal tract, (d) factors affecting the metabolic fate postabsorption (96).

Liver, especially beef liver is good source of folate. Folic acid is also naturally found in dried peas, dried beans such as black beans and kidney beans, germ, leafy and dark green vegetables such as spinach, lettuce, broccoli, fruits and fruit juices such as orange juice, tomato juice and also peanuts (81) (Table 2.2.5.1.).

In addition to food folate, ready-to-eat breakfast cereals contribute significantly to folate intake in the USA due to the majority of breakfast cereals in the United States marketplace contain about 100 μg per serving of folic acid (97).

Folic acid is an added ingredient in a various food products containing meal replacement and infant formulas, and ready-to-eat cereals, snack bars and cereal grain products (such as bread, pasta, flour, rice) (98). Table 2.2.5.2. shows the folic acid levels of some folic acid fortified foods in United States (81) (Table 2.2.5.2.).

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Table 2.2.5.1. Folat levels of some foods*

FOOD	Folat Content (μg/100g)
Liver	
Beef liver, cooked, braised, 3 ounces	185
Vegetables	
Cowpeas (blackeyes), immature, cooked, boiled, ½ cup	105
Spinach, frozen, cooked, boiled, ½ cup	100
Spinach, raw, 1 cup	60
Great Northern beans, boiled, ½ cup	90
Asparagus, boiled, 4 spears	85
Vegetarian baked beans, canned, 1 cup	60
Green peas, frozen, boiled, ½ cup	50
Broccoli, chopped, frozen, cooked, ½ cup	50
Broccoli, raw, 2 spears (each 5 inches long)	50
Lettuce, Romaine, shredded, ½ cup	40
Turnip greens, frozen, cooked, boiled, ½ cup	30
Germ	
Wheat germ, crude, 2 Tablespoons	40
Fruits	
Avocado, raw, all varieties, sliced, ½ cup sliced	45
Orange, all commercial varieties, fresh, 1 small	30
Cantaloupe, raw, ¼ medium	25
Papaya, raw, ½ cup cubes	25
Banana, raw, 1 medium	20
Fruit Juices	
Orange juice, chilled, includes concentrate, ¾ cup	35
Tomato Juice, canned, 6 ounces	35
Nuts	
Peanuts, all types, dry roasted, 1 ounce	40
Egg	
Egg, whole, raw, fresh, 1 large	25

Source: USDA,2003

Table 8.2.5.2. Folic acid levels of some folic acid fortified foods* *source: usda,2003*

FOLIC ACID FORTİFIED FOODS	Folic Acid (μg/100g)
Breakfast cereals fortified with 100% of the DV, ¾ cup	400
Breakfast cereals, fortified with 25% of the DV, ¾ cup	100
Rice,white,long-grain,parboiled,enriched,cooked, ½ cup	65
Bread, white, 1 slice	25
Bread, whole wheat, 1 slice	25

Recommendations for folate are given in the Dietary Reference Intakes (DRIs) developed by the Institute of Medicine of the National Academy of Sciences at 1998 (99).

The RDAs for folate are expressed in a term called the *Dietary Folate Equivalent*. Dietary folate equivalent (DFE) is a unit of expression for the folate DRIs (except the upper level) (99).

The Dietary Folate Equivalent (DFE) was developed to help account for the differences in absorption of naturally occurring dietary folate and the more bioavailable synthetic folic acid (99,100).

Table 2.2.5.3. lists the RDAs for folate, expressed in micrograms (μg) of DFE, for children and adults (99,100).

The RDA for all adult males and nonpregnant females ≥14 years is 400 μg DFE/day,respectively. It is determined that there is an additional requirement of 200 μg/day during pregnancy and 100 μg/day during breastfeeding (lactation) (99,100) (TABLE 2.2.5.3.)

There is insufficient information on folate to establish an RDA for infants. An adequate intake (AI) has been established that is based on the amount of folate consumed by healthy infants who are fed breast milk (99).

Table 2.2.5.4 gives the adequate intake for folate, in micrograms (μg) for infants (99).

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Table 2.2.5.3. Dietary reference ıntake values for folic acid by children and adult

Group	RDA (μg DFE/day)[a]
■ Children (male & female)	
1-3 years	150
4-8 years	200
■ Adolescent adult males & females	
9-13 years	300
14-18 years	400
19 to ≥70 years	400
■ Pregnancy	
<18 years	600
19-50 years	600
■ Lactation	
<18 years	500
19-50 years	500

[a]As Dietary Folate Equivalent (DFE)
***Sources*:** Institute of Medicine **(1998).** National Academy Press. Washington, DC ; Suitor CW & Bailey LB. **2000.** *J Am Diet Assoc.* 100, 88-94

Table 2.2.5.4. Dietary Reference Intake Values for Folic acid by Infant

Group	RDA (μg DFE/day)[a]
■ Infants (male & female)	
0-6 months	65
7-12 months	80

[a]As Dietary Folate Equivalent (DFE)
***Sources*:** Institute of Medicine **(1998).** National Academy Press. Washington, DC ; Suitor CW & Bailey LB. **2000.** *J Am Diet Assoc.* 100, 88-94

When expressed as DFEs, all forms of dietary folate, including folic acid in fortified products, are converted to an amount that is equivalent to food folate (88). The quantity of synthetic folic acid in the diet is firstly multiplied 1.7 times and this quantity is added to the μg of food folate (88). The conversion factor (1.7) was based on the observation that when folic acid is consumed with a meal, that is the usual case for a fortified product, then the added folic acid is about 85% available and food folate is abot 50% available. Therefore the ratio 85:50 yielded the multiplier of 1.7 in the DFE calculation (88).

A recent study provides confirming evidence for the validity of the use of the 1.7 multiplier to correct for the higher biovaliability of folic acid than the naturally occuring food folate (101) .

Folate is essentially nontoxic. It is reported that the upper limit of folate is 1000 μg. Deficiency produces megaloblastic anemia indistinguishable from that due to vitamin B_{12} deficiency. The appearance of this type of anemia is identical to that observed with vitamin B_{12} deficiency due to the common pathway shared by the folic acid and the active methyl cycles. One major difference between these two deficiencies is the absence of the neurological symptoms from lowered serum folic acid levels (88,94).

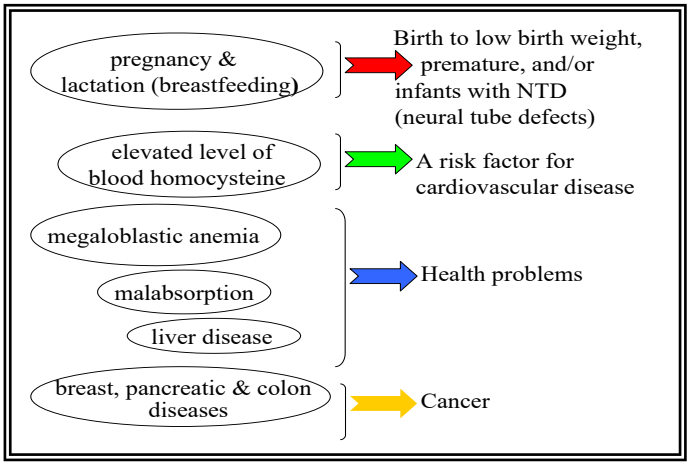

Figure 2.2.5.2. The major problems in folic acid deficiency

A deficiency of folate can occur when an increased need for folate is not matched by an increased intake, when dietary folate intake does not meet recommended needs, and when folate excretion increases. Medications that interfere with the metabolism of folate may also increase the need for this vitamin and risk of deficiency (102-104).

Folate requirements are increased in pregnant womans to meet the demands for DNA synthesis and one carbon transfer reactions in rapidly dividing fetal and maternal cells (88, 105). It is reported that the great risk of low infant birth weight, fetal growth retardation, premature baby, infants with neural tube defects develops if the folic acid intake is restricted or decreased during pregnancy (88, 102-105) (Figure 2.2.5.2.)

The neural tube forms in the developing embryo from the neural plate during the first 28 days postconception and develops into the spinal cord and its protrusion which encases the brain.

2.3. The interactions between most fortificated & dietary bioactive vitamins and genomics/vitamin-dependent genes: Nutritional, metabolic and medical aspects

The science of nutrigenomics is the study of how naturally occurring chemicals in foods alter molecular expression of genetic information in each individual. Scientific receptors exist for eiconosoids and vitamins (109-111).

Molecular studies have reported that dietary chemicals or their metabolites bind directly to nuclear receptors (transcription factors) and alter gene expression (TABLE 2.3.1.) (109).

Table 2.3.1. Some nuclear receptors[a,b]

Steroid	**Vitamin**	**Bile, cholesterol, xenobiotics**
Androgen (AR)	Retinoic acid (RAR)	Franosoid X (FXR)
Brassinosteroid (BR)	Retinoid X (RXR)	Liver X (LXR)
Estrogen (ER)	Vitamin D (VDR)	Pregnane X (PXR)
Thyroid	**Orphan receptors**	**Eiconosoid**
Thyroid (TXR)	>40 ligands uncharacterized	Peroxisome proliferator activated receptor (PPAR)

[a]Some receptors, like PPAR, have several related variants

[b] **Source:** From Fogg-Johnson & Kaput, **2003.** Nutrigenomics: An Emerging Scientific Discipline. *Food Technology*, 57 (4), 60-67 ; http://receptors.ucsf.edu/NR/

Vitamin D, through its daughter metabolite, the steroid hormone $1\alpha,25(OH)_2D_3$, functions in a manner homologous to that of the classical steroid hormones (112). According to general model, a steroid hormone is produced in an endocrine gland in response to physicological stimulus, then circulates in the blood, generally bound to the carrier DBP (vitamin D-binding protein), to target tissues where the hormone interacts with specific, high affinity intracellular receptors (112). It is known that using the advent of microarray analysis, several hundred genes are regulated by $1\alpha,25(OH)_2D_3$ (112-114).

By genetic defects in the α-tocopherol transfer protein, vitamin E deficiency is caused. Genetic defects in α-TTP are associated with a characteristic syndrome, ataxia with vitamin E deficiency which characterized neurological abnormalities. It is shown that a dose of 800-1,200 mg/day is usually adequate to prevent further deterioration of neurological functions (8). Vitamin E deficiency is also caused by genetic defects in lipoprotein synthesis. Daily doses of 100-200 mg/kg, or ~5-7 g of RRR-α-tocopherol are recommended regarding the preventing dose for genetic defects in lipoprotein synthesis (8).

Figure 2.3.1. shows that genes associated with folate and clinical conditions (115). It has studied that the administration of 9-cis retinoic acid to such animals brought about no accumulation of the CRBPII mRNA (116). So, in spite of vitamin A deficiency, oral administration of corn oil, but not 9-cis retinoic acid, caused an increase in jejunal CRBPII mRNA level. It has reported the CRBPII gene expression in rat jejunum may be regulated predominantly by dietary Fas, but little by dietary retinoids (117).

It is determined that the physicological action of retinoids is expressed through nuclear receptors which can bind retinoids and regulate the expression of various genes. All *trans*-retinoic acid and 9-cis retinoic acid which are active metabolites of vitamin A are utilized as ligands for nuclear receptors (RAR, RXR), that results in regulation of the expression of various genes at the transcription level (116-117).

The downstream metabolites of retinol, all-trans and 9-cis retinoic acids are the bioactive components that bind and activate their cognate nuclear receptors to regulate target genes (118). It has provided detailed information on the specific nuclear receptors, coactivators and chromatin modification events that occur when vitamin A is deficient and, therefore, retinoids are not available to activate the nuclear retinoid-signaling cascade (118).

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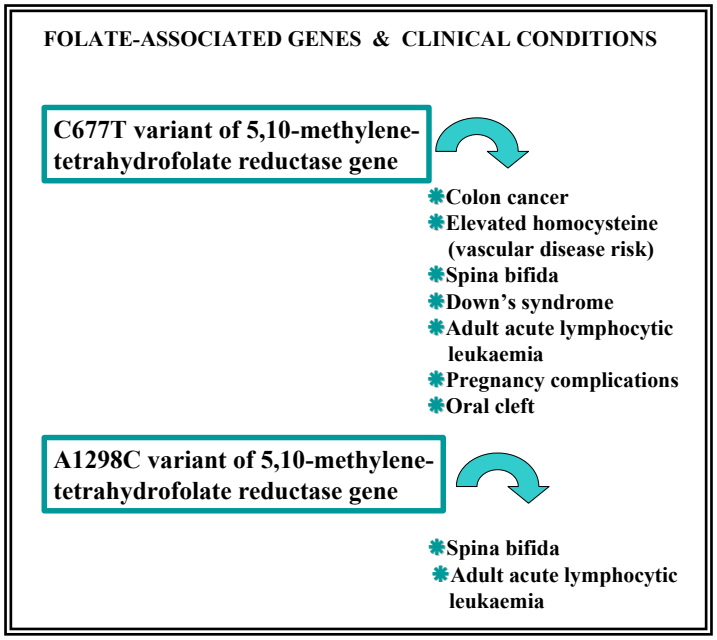

Figure 2.3.1. Genes associated with folate and clinical conditions

Therapeutic effects in hyper proliferative and inflammatory diseases, such as acne, psoriasis, photoaging and neoplasia's are accomplished by the abilities of the biologically active form of vitamin A, retinoic acid and its synthetic analogs to regulate complex programs of gene expression in target cells via binding to nuclear receptors, that are ligand-dependent transcription factors (119).

It is reported that the insulin-like growth factor-I receptor (IGF-IR) has an crucial role in colorectal cancer development and progression (120). Folic acid is a chemopreventive agent whose deficiency has been linked to an enhanced colon cancer risk. IGF-IR displays a potent anti-apoptotic activity and is overexpressed in primary tumors and colon cancer-derived cell lines (120). The regulation of IGF-IR gene expression by folic acid was assessed utilizing RT-PCR, transient transfections and chromatin immunoprecipitation assays (120). It is also determined that folic acid deficiency may lead to increased IGF-IR gene expression, with ensuing pathological activation by endocrine and/or autocrine/paracrine IGF-I (120).

It is reported that the folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring (121).

A. *1α,25-dihydroxyvitamin D_3 (1α,25(OH)$_2$D$_3$) analogs inhibit growth* in vitro *and* in vivo *of cells derived from a variety of tumors (122). It has examined that the effects of 1α,25(OH)$_2$D$_3$ and its analog EB1089 on proliferation and target gene regulation of human head and neck squamous cell carcinoma (SCC) lines SCC4, SCC9, SCC15, and SCC25 (1229. The anti proliferative effects of 1α, 25(OH)$_2$D$_3$ and EB1089 in SCC25 cells were analyzed by screening more than 4,500 genes on two cDNA microarrays, yielding 38 up-regulated targets, containing adhesion molecules, growth factors, kinases, and transcription factors (122).*

B. *Increased* NCoR1 *mRNA is a novel molecular lesion in breast cancer cells, that acts to suppress responsiveness of VDR target genes, resulting in 1α,25(OH)$_2$D$_3$ resistance and seems to be particularly associated with estrogen receptor negativity (123).The above-mention lesion provides a novel molecular diagnostic and can be targeted via combinations of vitamin D_3 compounds and low doses of trichostatin A (TSA; histone deacetylation inhibitor) (123).*

1α,25-Dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$] regulates calcium homeostasis and controls cellular differentiation and proliferation (124). The vitamin D receptor (VDR) is a ligand-regulated transcription factor that recognizes cognate vitamin D response elements (VDREs) formed by direct or everted repeats of PuG(G/T)TCA motifs separated by 3 or 6 bp (DR3 or ER6) (124). It has identified that direct 1,25(OH)$_2$D$_3$ target genes by combining 35,000+ gene microarrays and genome-wide screens for consensus DR3 and ER6 elements, and DR3 elements containing single nucleotide substitutions (124).

It has reported the interaction of vitamin D and retinoid receptors on regulation of gene expression (125). Vitamin D and retinoic acid (RA) receptors (VDRs and RARs, respectively), bind as heterodimers with the retinoid X receptor (RXR) to hormone response elements (HREs) in target genes (125).

Vitamin D-influenced gene expression by a ligand-independent, receptor-DNA complex intermediate has reported (126). It has shown that vitamin D receptor (VDR), derived from extracts of the small intestines of

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vitamin D-deficient rats, is capable of binding a vitamin D response element (DRE) by an electrophoretic mobility-shift analysis (126).

It has found that $1,25(OH)_2D_3$ most substantially increased the expression of the insulin-like growth factor binding protein-3 (*IGFBP-3*) gene (127). Additionally, was found that, some of the key genes regulated via $1,25(OH)_2D_3$ are also androgen-responsive genes (127). The up-regulation of *IGFBP-3* gene has been shown to be important in $1,25(OH)_2D_3$-mediated inhibition of LNCaP cell growth.The putative $1,25(OH)_2D_3$ target genes appear to be involved in various of cellular functions containing growth regulation, differentiation, membrane transport, cell-cell and cell-matrix interactions, DNA repair, and inhibition of metastasis (127).

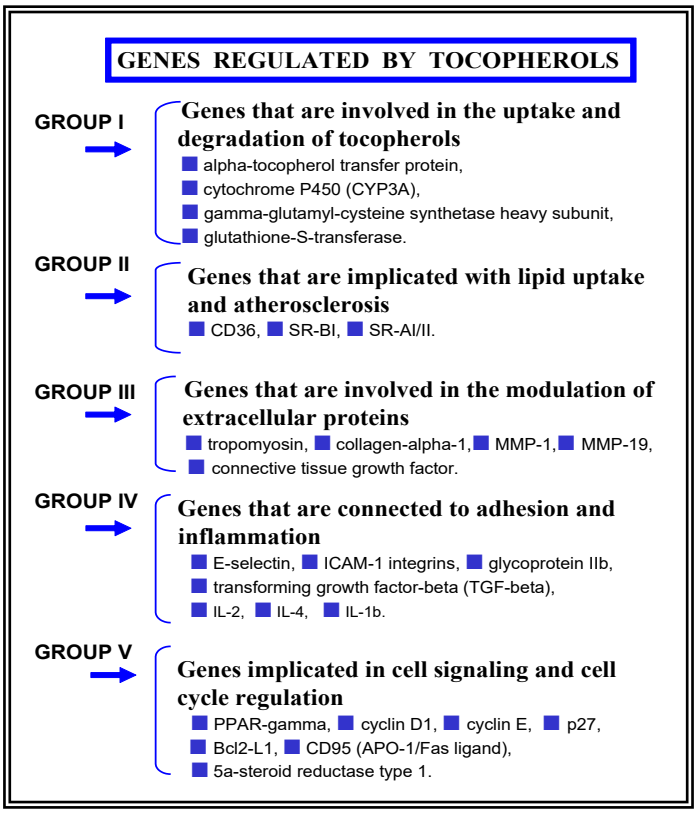

Figure 2.3.2. Genes regulated by tocopherols.

Several genes are regulated by tocopherols partly due to the effects of tocopherol on these two kinases (protein kinase C and phosphatidylinositol 3-kinase), but also independently of them (128). Genes regulated by tocopherols can be divided in five groups (128). Figure 2.3.2. shows the several genes regulated by tocopherols. Group I, group II, group III, group IV and group V of genes contains genes that are involved in the uptake and degradation of tocopherols, genes that are implicated with lipid uptake and atherosclerosis, genes that are involved in the modulation of extracellular proteins, genes that are connected to adhesion and inflammation, genes implicated in cell signaling and cell regulation, respectively (Figure 2.3.2.).

The regulation of genes via tocopherols has been associated with protein kinase C owing to its deactivation by α-tocopherol and its contribution in the regulation of a number of transcription factors (NF-kappaB, AP1) (128).

It is known that T cells are vulnerable to age-associated changes. Vitamin E has been shown to improve T cell functions in the old. It is reported that gene expression profiles of T cells to better understand the underlying mechanisms of age and vitamin E-induced changes in T cell function (129). According to performed experiments with young and old C57BL mice, 500 ppm of vitamin E supplementation for 4 wks resulted in higher expression of genes involved in cell cycle regulation (*Ccnb2*, *Cdc2*, *Cdc6*) in old T cells. Gene expression profiles of T cells were assessed utilizing microarray analysis with/without anti-CD3/anti-CD28 stimulation (129). It has found that vitamin E supplementation resulted in higher up-regulation of IL-2 expression in young and old T cells whereas lower up-regulation of IL-4 expression in old T cells following stimulation and also resulted in higher up-regulation of IL-2 expression in young and old T cells whereas lower up-regulation of IL-4 expression in old T cells following stimulation (129). It has suggested that aging has significant effects regarding the gene expression associated with signal transduction, transcriptional regulation and apoptosis pathways in T cells and vitamin E has a significant impact concerning the gene expression associated with cell cycle and Th1/Th2 balance in old T cells (129).

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