Chapter 7

Marine lipophilic compounds

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Marine lipids have excellent potential as bioactive compounds as they possess advantageous physiological effects, with medicinal characteristics and added health benefits such as anticancer or anti-inflammatory activity. The marine world, due to its phenomenal biodiversity, is a rich natural resource of many biologically active compounds such as poly unsaturated fatty acids (PUFAs), sterols, proteins, poly saccharides, antioxidants and pigments. Many marine organisms live in complex habitats exposed to extreme conditions and, in adapting to new environmental surroundings, they produce a wide variety of secondary (biologically active) metabolites which cannot be found in other organisms. Marine lipids can be derived from a vast array of sources, including marine plants, microorganisms, and sponges. Earlier this century, Thomas A. Edison predicted that "the doctor of the future will give no medicine, but will interest his patients in the care of the human frame, in diet, and in the cause and prevention of disease." In the years ahead physicians and patients alike embrace Mr. Edison's prediction and look to natural sources for healing and wellness. The consumption of diets rich in seafood is associated with a reduced risk of vascular diseases and certain cancers.

Squalene

Epidemiological studies (Das, 2000; Pedersen *et al.*, 1999) have revealed that Greenland Eskimos and Japanese with diets rich in fish oil show low incidence of ischemic heart disease as compared with European and North American populations. Prospective studies (Okuda *et al.*, 2005; Holub and Holub 2004) show that there is an inverse relation between fish intake and mortality from coronary heart disease. It is hypothesized that the highly

unsaturated fatty acids, eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) present in substantial quantities in Mediterranean diet are the active components responsible for this beneficial effect (Jude, *et al.*, 2006). Squalene, an isoprenoid molecule, which also comprises a major portion in Mediterranean diet, is present in large quantities in deep-sea shark liver oil and in smaller amounts (0.1-0.7%) in palm oil, wheat-germ oil, olive oil, and rice-bran oil (Liu *et al.*, 1976) and has been reported to possess antilipidemic, antioxidant and membrane-stabilizing properties (Qureshi *et al.*, 1996; Ko *et al.*, 2002; Ivashkevich *et al.*, 1981). Shark liver oil is a natural source for squalene. Deep-sea shark livers generally have high level of hexa-unsaturated isoprenoid alkene, squalene (Summers, 1987). The liver is the principal site of lipid storage and high content of low-density squalene (specific gravity 0.86 at 25°C) which is believed to provide hydrostatic lift and enable the shark to maintain neutral buoyancy (Hayashi and Takagi, 1981). Recently squalene has been extracted and purified from Amaranth seeds, which contain about 5.1-7.7% squalene (He *et al.*, 2002).

The chemical structure of squalene is that of a linear triterpene (Figure1). The structural formula of squalene was certified and fixed by Dr. Calour, a Swiss chemist and Nobel peace prize awardee. The term squalene was coined in 1916 after the discovery of high concentrations of the $C_{30}H_{50}$ hydrocarbon in squaloid shark liver oil; some early reports also refer to squalene as spinacene (Channon, 1926; Tsujimoto, 1916). Chemically it is known as 2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22- hexaene having a molecular weight of 410.70. The triterpene, squalene ($C_{30}H_{50}$) is an isoprenoid compound containing six isoprene units (Fig. 1).

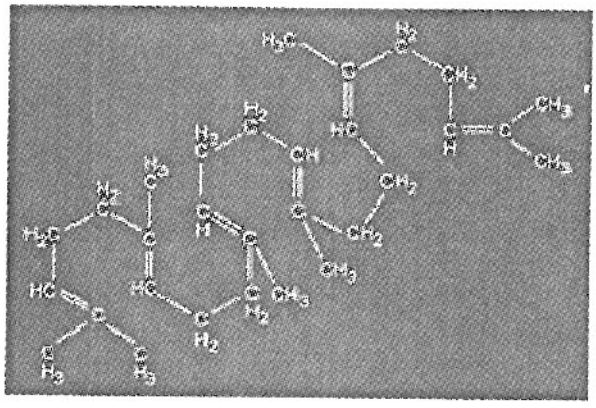


Fig. 1 Chemical structure of squalene

Significant amounts were later found in olive oil and olive leaves (Murkovic *et al.*, 2004; Ollivier *et al.*, 2003; Smith, 2000). In addition, squalene is present in other diverse sources such as wheat germ oil, rice bran oil, carrots, *Phycomyces blakesleeanus* mold, alfalfa, elderberry, and lettuce (Deiana *et al.*, 2001; Kelly, 1999). Moreover, squalene is a main component of human sebum and a precursor of cholesterol biosynthesis (Huang *et al.*, 2009).

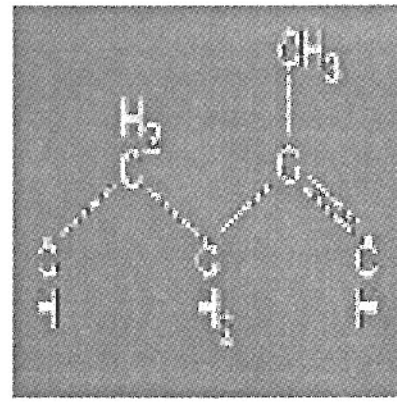


Fig. 2 Structure of isoprenyl unit of squalene

Squalene is the principal hydrocarbon of human surface lipids amounting up to 11% of total surface fat. It is also seen in dermoid cysts, cerumen, hair fat and sebum. Squalene is usually bound to sterol-carrier proteins in hepatocytes. In addition to the contribution to cholesterol biosynthesis, a portion of the hepatic squalene pool is secreted *via*. bile. In fact, the squalene levels are much higher in bile than in plasma, and in subjects on squalene-free diets, squalene appears also in feces. Squalene is used as bactericide, intermediate in the manufacture of pharmaceuticals, organic colouring matter, rubber chemicals, aromatics and surface-active agents. Squalene is now extensively used as an additive in pharmaceutical preparations and certain foodstuffs. A health food called squalene powder is popular. It is prepared by adding proteins, carbohydrates, flavoring agents etc. to squalene. This is soluble in water and stable during storage. Another important use is in finishing natural and artificial silk to which it imparts an unusually brilliant sheen. It is also an excellent lubricant and also used widely in filling certain types of thermometers. As it contains a number of isoprene

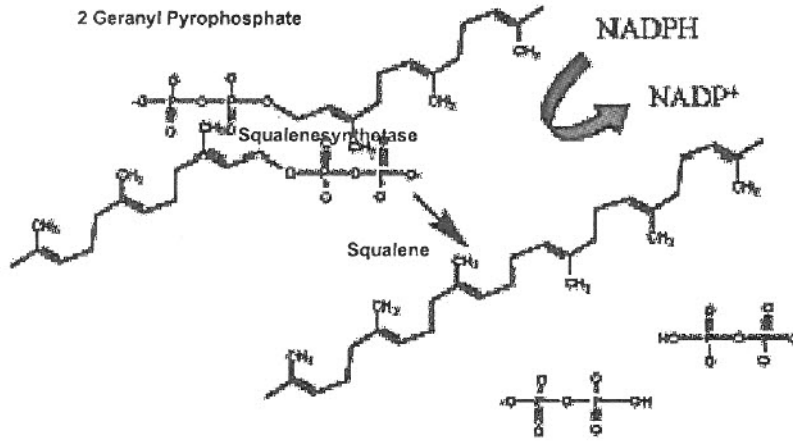


Fig. 3 Synthesis of squalene

units, it is an excellent raw material for preparation of various chemicals, particularly aromatics, by condensation and cyclisation. It is also used in synthesis of a number of steroid hormones of immense use to the mankind.

Squalene is synthesized from acetate and is metabolized to cholesterol in liver. The endogenous synthesis of squalene begins with the production of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA). The initial reduction of HMG CoA (a niacin-dependent reaction) results in the formation of mevalonate. The enzyme involved in this reduction, HMG CoA reductase, has been the target of a class of cholesterol-lowering drugs.

Mevalonate is then phosphorylated in three stages (*via*. magnesium-dependent enzymes) and finally decarboxylated to form delta 3-isopentenyl diphosphate, the donor molecule for all poly prenyl compounds. Successive additions of prenyl groups result in the formation of the 15-carbon farnesyldiphosphate. Two molecules of farnesyldiphosphate are then enzymatically joined and reduced (niacin dependent) resulting in the formation of squalene. After its biosynthesis, squalene can be transported to other areas of the body for incorporation into tissues or it can be further metabolized, resulting in the eventual formation of cholesterol and its steroid metabolites.

Physiological Properties of Squalene

Anticarcinogenic property: Squalene is reported to inhibit aberrant hyper proliferation, a cellular marker associated with pre-neoplastic transformation (Katdare *et al.*, 1997). Newmark (1997) has proposed that the relatively high squalene contents of olive oil is a contributing factor for the epidemiological observation of reduced risk for several cancers associated with olive oil intake. Studies on and its related substances like Vitamin A, E and K, β-carotene, Ubiquinone (CoQ10) and phytol indicate that the squalenes as well as the related compounds are capable of suppressing the growth of tumor cells (Tomitu, 1983). Studies on squalene and its interaction with anti-cancerous agents showed that among the tested agents, squalene is the most effective in potentiating the anti-tumor activity of bleomycin (Nakagawa *et al.*, 1985). Investigation of Ikekawa *et al.* (1986) on anti-tumor activity of squalene and its related compounds also indicate that they possess a high tumor growth inhibitory effect against sarcoma-180. Squalene has also been found to be an efficient chemo-preventive agent against lung metastasis in mice bearing lung carcinoma by oral administration (Ikekawa *et al.*, 1986). Murakoshi *et al.* (1992) have reported that squalene is able to inhibit the effect of the tumor promoting agent 12-o-tetradecanoyl-phorbil-13-acetate (TPA) in mouse skin carcinogenesis. Desai *et al.* (1996) have reported both squalene and Roidex, a squalene-containing compound, partially prevent the development of chemically induced cancer and cause regression of some already existing tumors in a mouse skin model. Rao *et al.* (1998) have also suggested that squalene, as a constituent of olive oil is particularly responsible for its chemoprotective effect.

Anti radiation property: Squalene has been reported to have some protective action on the effect of radiation on body. Storm *et al.* (1993) have reported that administration of squalene at 2% of the diet to mice for 14 days pre-and 30 days post-exposure to lethal whole-body

gamma irradiation resulted in cellular and systemic radioprotection. Squalene treatment has been reported to significantly prolong survival time as compared with control fed mice, subsequent to exposure to lethal doses of radiation. *In vitro* experimental evidence indicates that squalene is a highly effective oxygen-scavenging agent. Squalene is not easily susceptible to peroxidation and appears to function in the skin as a quencher of singlet oxygen, protecting human skin surface from lipid peroxidation due to exposure to ultraviolet and other sources of oxidative damage.

Antioxidant property: Ko *et al.* (2002) has identified squalene in extracts of *Terminalia catappa* leaves and seeds. When these extracts were applied for antioxidative characterization by supplementation in an iron/ascorbate system with linoleic acid, they exhibited potent antioxidative and free radical scavenging activities. The rate constant of quenching of singlet oxygen by squalene is much larger than those of other lipids in human skin surface and in 3,5 dibutyl-4-hydroxy toluene (BHT) (Kohno *et al.*, 1995). Reports of Passi *et al.* (2002) indicates that squalene, vitamin E and coenzyme Q_{10} (Co Q_{10}) are increasing from childhood to maturity in skin surface lipids and decreasing again significantly in old age, suggesting that these antioxidants protect the body against exogenous oxidative insults and aging. Owen *et al.* (2000) have reported that high consumption of extra-virgin olive oil, which is rich in phenolic antioxidants, squalene and oleic acid afford considerable protection against cancer, coronary heart disease and aging by inhibiting oxidative stress.

Detoxification property: Squalene can act as a "sink" for highly lipophilic xenobiotic assisting with their elimination from the body. Since it is a non-polar substance, it appears to have the highest affinity for unionized drugs. Reports of Kamimura *et al.* (1992) suggest that squalene can be a good candidates as antidote to reduce the toxicity of drug ingested accidentally at a high dose by enhancing the drug elimination from the body. In animal experiments, squalene increases the fecal excretion of theophylline and strychnine in mice, indicating that squalene stimulates elimination of more neutral (theophylline) or basic (strychnine) drugs, which should be present in unionized form in intestinal lumen than that of acid drugs. Squalene stimulates the fecal excretion of 2, 4, 5, 2', 4', 5'-hexachlorobiphenyl (6-CB) in rats (Richter *et al.*, 1982). Addition of 8% squalene to the diet 2, 6 and 15 weeks after dosing results in five-fold increase of daily 6-CB excretion in feces independent of the time of beginning of the treatment. It shows that 6-CB elimination can be enhanced by oral treatment with squalene even long time after the uptake of the poison.

Antilipidemic property: Although oral administration of squalene appears to decrease hepatic and serum levels of plant sterols consistently, its impact on cholesterol metabolism in human is less clear. Squalene consumption of 0.86 g/day has been found even to reduce serum cholesterol (Chan *et al.*, 1996). The compensatory mechanisms to prevent the increase of serum cholesterol during moderate long-term squalene consumption are reduced HMG-CoA reductase activity and increased fecal elimination of cholesterol as cholesterol itself and as bile acids (Strandberg *et al.*, 1990). Earlier study by Qureshi *et al.* (1996) on the effect of Amaranth oil (which contains squalene and tocotrenol) on cholestergenesis in six-week-

old female chicks shows that serum total cholesterol and LDL cholesterol are decreasing without altering the HDL cholesterol levels. Brakken *et al.* (1961) have reported that squalene administration to one-day-old chicks lowers the serum cholesterol levels. Squalene feeding significantly increases fecal excretion of cholesterol, its non-polar derivatives and bile acids, suggesting that, although cholesterol synthesis probably increased by as much as 50%, fecal elimination was also up regulated resulting in no significant difference on serum cholesterol concentrations (Strandberg *et al.*, 1989).

Replacement of dietary triglyceride by squalene is reported to result in 50% reduction in the absorption of cholesterol in rats (Richter and Schafer, 1982b). The double blind placebo-controlled study conducted by Chan *et al.* (1996) to compare pravastatin (10mg), a hypolipidemic drug, and squalene (860mg) either alone or in combination for the treatment of hypercholesterolemia in 102 patients received either treatment or placebo for 20 weeks shows that pravastatin is more effective than squalene in reducing total cholesterol, LDL cholesterol and triglyceride and in increasing levels of HDL cholesterol. But the combination of both pravastatin and squalene is more effective in reducing total and LDL cholesterol and increasing HDL cholesterol than that of pravastatin alone, which indicates that squalene is a prudent dietary addition for individuals on similar cholesterol lowering drugs.

Oxygen carrying property: Squalene revitalizes weakened body cells and helps to revive cell generation. Its chief attribute is the protection of cells from oxidation reactions. The human body has about six billion oxygen reliant cells. Oxygenation to the cells promotes good health to the most basic level of life (Gregory and Kelly, 1999). Squalene helps to clean, purify, and detoxify the blood from toxins, facilitating circulation. It cleanses the gastrointestinal tract and kidneys, causing better bowel movement and urination. Many diseases are cured if the blood is purified, by supplementing squalene (Gregory & Kelly, 1999). Squalene carries oxygen in the cellular level, causing further improvement in organ function through cellular metabolism, preventing the acidotic cell syndrome where cells become acidic, deteriorate and die due to lack of oxygen (Yokota, 1995).

Other actions: Squalene's terpene gives a sterilizing effect, combating the growth of various microorganisms such as coliform bacilli, dysentery bacilli, *Micrococcus pyocynanel*, staphylococcus, hemolytic streptococcus, and *Candida albicans* (Masuda *et al.*, 1982). Squalene naturally increases male potency and vitality through a better body. It also helps regulate the female menstrual cycle and improves irregular and abnormal cycles (Rukmini and Raghuram, 1989). Sebum provides normal lubrication for hairy and non-hairy skin. It keeps skin supple and forms a protective bacterial and fungicidal coating of skin and in pilosebacious apparatus. This fatty cover helps to keep moisture on the skin surface. Squalene occurs naturally in human sebum (Passi *et al.*, 2002). Thus, it is essential to have adequate dose of squalene to maintain the integrity of skin and also acts as an anti-ageing compound. Squalene is now used as an immunoprotector (Abn and Kim, 1992). Squalene reduces various aches and pains, helps body organ such as the kidney, liver and gall bladder and digestive system to function properly, helps hemorrhoids to shrink and curb obesity. It also acts as

relaxant, giving added vigor and vitality without the hyper-activity associated with other food supplements, generates hair and smoothens skin. It exhibits penetrating action with immediate effect on topical applications and helps to prevent various kinds of disease and speeds up the healing process in most conditions of ill health (Gopakumar, 2002).

Poly unsaturated Fatty Acids (PUFA)

Chemically poly unsaturated fatty acids (PUFA) belong to the class of simple lipids and have two or more double bonds. The location of the first double bond, counted from the methyl end of the fatty acids, is designated by the omega or n- number. Two broad categories of PUFAs that are of concern with respect to cardiovascular homeostasis are E-PUFAs (essential PUFAs) and NE-PUFAs (non essential PUFAs). The essential PUFAs must be provided in the diet as they can't be synthesized from simple carbon precursors in mammalian organisms. The presence of a high proportion of highly poly unsaturated fatty acids - those having more than four double bonds- makes the fish oil unique in nature. While fish oils contain primarily ω-3 series of fatty acids, vegetable oils contain mainly ω-6 series of fatty acids. The most important PUFA present in fish are eicosapentaenoic acid (EPA, C20:5n-3) (Fig. 4) and docosahexaenoic acid (DHA, C22:6n-3) (Fig. 5). These belong to ω-3 series of fatty acids. They cannot be synthesized by the body but are needed, particularly, for the formation of the retina and of the brain. α-Linolenic acid can be converted to EPA and DHA in the human body. However, the extent of this conversion is not precisely known and, at best, very limited.

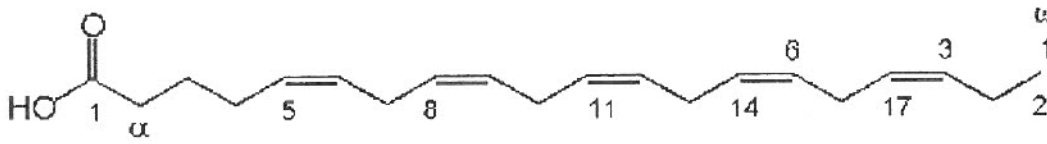


Fig. 4. Chemical structure of eicosapentaenoic acid (EPA)

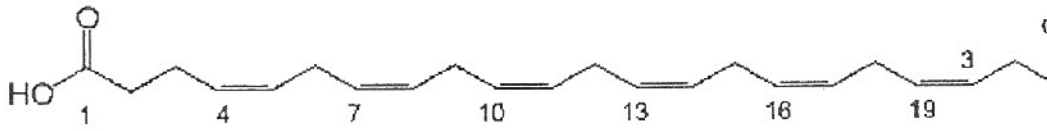


Fig. 5. Chemical structure of docosahexaenoic acid (DHA)

Depending on nutritional intake, ω-3 fatty acids are incorporated in the phospholipid pool of cellular membranes and replace the ω-6 fatty acids, thereby increasing membrane fluidity and influencing lipid mediator and cytokine production. ω-3 fatty acids affect biophysical characteristics of cellular membranes by alteration of the membrane phospholipid composition and the content of cholesterol, which improves membrane fluidity. The associated increase in the deformability of blood cells might account for improvement of blood rheology after fish oil intake. Furthermore, ω-3 fatty acids modify the function of

membrane-linked enzyme systems, signal transduction and receptor functions. LDLs bind many fatty acid molecules and nearly half of them are PUFA. Linoleic acid accounts for 86% of PUFA and is mainly (65%) contained in the cholesteryl esters, whereas arachidonic acid accounts for 12% and is mostly (68%) found in the phospholipids. Docosahexaenoic acid is present in trace amounts, mainly in phospholipids (Esterbauer *et al.*, 1989).

Essential linoleic acid (C18:2 ω-6), which is present in the food, is converted either into arachidonic acid of class ω -6 (whose first double bond is located at the 6th carbon atom of the CH_3-end of the hydrocarbon chain) or, by a shunt via γ-linoleic acid (C18: 2n-3), into n-3 PUFA - eicosapentaenoic (C20:5, EPA) and docosahexaenoic (C22:6, DHA) acids. ω-3 PUFA can directly inhibit the metabolism of n-6 PUFA, especially at the desaturation stage (Garcia and Holman, 1965; Sprecher, 1981). Despite certain structural distinctions, arachidonic acid, on the one hand, and EPA and DHA, on the other, compete naturally with one another for the same enzymes and are synthesized from the same precursors - linoleic and linolenic acids. These FAs are metabolized *via*. the arachidonic acid pathways. Besides its ability to incorporate into membrane phospholipids, arachidonic acid serves as a substrate for two important enzymes - lipoxygenase, which gives rise to leukotrienes, and cyclooxygenase, which produces endoperoxides, the substrates for platelet synthetases forming thromboxanes, and endothelial synthetases which make prostacyclins. EPA and DHA effectively compete with n-6 PUFA for cyclooxygenases initiating the synthesis of prostaglandins with changed properties. The formation of metabolites *via.* lipoxygenase and cyclooxygenase routes (which minimizes the risk of clot formation) is one of the most beneficial effects of n-3 PUFA-enriched diets for the cardiovascular system.

Fish oil and PUFA

Fish and fish oils contain very-long chain and highly unsaturated n-3 PUFA such as eicosapentaenoic acid and docosahexaenoic acid. Fish oils reduce the synthesis of chylomicrons by the intestine and/or increase their removal from circulation, thus decreasing post prandial lipemia (Harris *et al.*, 1988). Chylomicron remnants are selectively cleared after the ingestion of n-3 PUFA (Bergeron and Havel, 1997). In this regard, Lambert *et al.* (1995) reported that labeled [1-^{14}C] oleate and [1,2-^{3}H] cholesterol in chylomicrons remnants derived from fish oil are incorporated into phospholipids more efficiently than those derived from olive, corn or palm oil remnants and that fish oil remnants are metabolized more rapidly than palm oil remnants. The hypolipidemic effect of fish oil is stronger on hyperlipidemic patients than on normal subjects (Harris, 1989). Cholesterol concentration in plasma is decreased by fish oil and by n-3 PUFA in patients with Type V hyperlipidemia who do not tolerate any other type of dietary fat (Schmidt *et al.*, 1993; Harris, 1989).

PUFA and lipid profile

Mono unsaturated fatty acid (MUFA) and poly unsaturated fatty acid-rich diets decrease the levels of total plasma cholesterol and LDL-cholesterol and increase HDL-cholesterol in healthy normolipidemic subjects (Grundy, 1986; Grundy and Denke, 1990) and in mouse

models of atherosclerosis (George *et al.*, 2000). Although the ingestion of n-3 PUFA has a lower effect than MUFA on plasma cholesterol and on LDL and HDL cholesterol levels (Keys *et al.*, 1986; Harris, 1989; Harris *et al.*, 1983). The slight effect of fish oil on plasma LDL and HDL, as against the decrease in very low-density lipoproteins and triacylglycerol concentrations, is the result of factors such as the smaller very low-density lipoprotein particle produced, which is more likely to be converted to LDL (Huff and Telford, 1989), by the direct effect on the synthesis of LDL by the liver and by lowering the saturated fat intake. These effects depend largely on the dose and type of n-3 PUFA content of fish oil used. Altogether PUFA diet decreases triacylglycerol level, increases the HDL/LDL-cholesterol ratio and decreases the total cholesterol/HDL-cholesterol ratio, thus reducing the risk of atherosclerosis and coronary artery disease.

PUFA and Coronary Heart Disease

Fish oils contain a large proportion of long chain *n*–3 fatty acids and supplementation with these materials has been recommended for patients with ischaemic heart disease (Kinsella, 1987) and in disorders such as psoriasis (Bittine *et al.*, 1988). Now concern has been raised over the fact that the *n*–3 fatty acids present in fish oils are more susceptible to degradation by free radical species (Wills, 1985). Hu *et al.* (1989) have shown that consumption of fish oils exacerbates the susceptibility of the tissues to free radical-mediated lipid peroxidation *in vitro*. PUFA are particularly susceptible to lipid peroxidation and modification of the poly unsaturated content of the myocardial membranes would be expected to influence their susceptibility to lipid peroxidation. Rhodes *et al.* (1994) reported that n–3 PUFA supplementation in humans did not have a deleterious effect on skin damage caused by exposure to ultraviolet light, a process associated with epidermal lipid peroxidation. Van den Berg *et al.* (1991) reported an increased n–3 fatty acid content of red blood cells from fish oil-fed rabbits caused an increase in *in vitro* lipid peroxidation, but decreased the rate of haemolysis. They proposed that the increased *n*–3 fatty acid content following fish oil supplementation in the erythrocytes, acted as an oxidizable buffer, competing for a limited supply of free radicals, which were generated under circumstances of oxidative stress and so preventing the peroxidation of other molecules.

Observational studies indicate that intake of fish is associated with less fatal coronary heart disease in several populations. Bang *et al.* (1980) suggested 37 years ago that the low occurrence of fatal coronary heart disease in Inuits (Eskimos) could be related to their high intake of marine ω3 PUFA (10-14g/day). This ecological study was the basis for the hypothesis that consumption of marine ω3 PUFA could protect against coronary heart disease. Fish consumption was inversely related with fatal coronary heart disease and sudden cardiac death -but not with non-fatal myocardial infarction- in a dose-dependant way, where each 20 g/day increase in fish intake was associated to a 7% lower risk of fatal coronary heart disease (He *et al.*, 2004). Recently, in a large prospective randomized clinical trial of 11,324 patients with recent myocardial infarction, administration of 850 mg EPA plus DHA daily, in addition to pharmacological treatment, led to a 45% reduction in mortality at 42 months

(Marchioli *et al.*, 2002).

Burr *et al.* (1989) demonstrated that mortality following myocardial infarction was reduced by 29% after two years in a group of men who were advised to eat oily fish at least twice weekly as compared to others who had not received any recommendation. Interestingly, this decrease in mortality was not associated with reduced ischemic heart disease or total cholesterol levels, and thus may be related to protection of the heart muscle itself. In a 6 week study in 188 patients scheduled for carotid endarterectomy, it is found that EPA and DHA were incorporated into carotid plaques in patients randomized to n-3 PUFA, and those patients had less infiltration with macrophages and a thicker fibrous cap than the controls (Thies *et al.*, 2003). These findings suggest that marine n-3 PUFA may change the composition of atherosclerotic plaques making them less vulnerable to rupture, a very important finding that awaits confirmation.

Major beneficial effects of marine n-3 PUFAs in coronary heart disease are relating to their ability to decrease level of triglycerides, platelet reactivity, leukocyte reactivity and blood pressure and their antiarrhythmic properties (Schmidt *et al.*, 2006). Dietary n-3 PUFA rapidly incorporate into cardiac phospholipids (predominantly, phosphatidyl ethano lamines and phosphatidyl cholines) which constitute up to 90% of the overall phospholipids pool of the myocardial membranes (Swanson and Kinnsella, 1986; Nalbone *et al.*, 1988; Nalbone *et al.*, 1989). It would be natural to expect that the cardioprotective effect of n-3 PUFA is not confined to just reducing the risk of clot formation but is manifested also at the cardiomyocyte level as the alteration of the fatty acid composition of the membrane structures.

PUFA and Diabetes

In streptozotocin-diabetic rats, long-term ω-3 PUFA supplementation has been shown to prevent diabetic heart muscle disease (Black *et al.*, 1989). In neonatal cardiomyocytes cells, arrhythmia caused by agents such as high extra cellular calcium, ouabain, isoproterenol or lysophosphatidyl choline was prevented by exogenous EPA in the free form (Calder, 2004). As removal of free EPA with added bovine serum albumin quickly reversed this protective effect (Kang and Leaf, 1994), it was suggested that the free carboxylic group of ω-3 PUFA modulates ion channels, especially the calcium and sodium channels on the cardiomyocyte membrane to prevent arrhythmia (Calder, 2004). It is possible that through similar mechanisms, EPA could prevent calcium overload in the diabetic heart, which is known to induce mitochondrial pore transition leading to cytochrome c release and cardiomyocyte apoptosis (Oliveira *et al.*, 2003). Interestingly, exogenous DHA supplementation has also been demonstrated to correct calcium homeostasis and mitochondrial dysfunction in diabetic cardiomyocytes (Ovide-Bordeaux and Grynberg, 2004). As inhibition of protein kinase C (PKC) is associated with a reduction in reactive oxygen species generation, DHA has been shown to inhibit generation of superoxide from neutrophils (Arita *et al.*, 2001). It is possible that DHA, through its prevention of PKC activation and oxidative stress, could limit premature apoptosis of diabetic cardiomyocytes. *In vitro*, long-chain n-3 PUFAs decrease myocyte

excitability and reduce cytosolic calcium fluctuations *via*. inhibition of Na^+ and L-type Ca^{2+} channels, supporting a potential antiarrhythmic effect of these fatty acids (Leaf, 2001; Leaf *et al.*, 2003).

PUFA and Cancer

Many trials using fish oil or PUFAs from fish oil as diet shows promising results in the area of cancer treatment. In rats, linoleic acid, a precursor of arachidonic acid in tissues, increases the size and number of tumours whereas EPA and DHA decrease both (Galli and Butrum, 1991). It is suggested that the potential of n-3 fatty acids to prevent recurrence and metastases of mammary cancer when used in adjuvant therapy is associated with a (n-6) to (n-3) ratio < 2:1(Cowing and Saker, 2001). In humans, dietary (n-3) fatty acid treatment offers possibilities in malignant diseases (Anti *et al.*, 1992).

In contrast, low α-linolenic acid (precursor of EPA and DHA) levels in mammary adipose tissue are associated with an increased risk of breast cancer in women (Klein *et al.*,2000). In patients with prostate cancer, fish intake was inversely related to cancer (Terry *et al.*, 2001). In the great majority of colon adenocarcinomas taken from humans, COX-2 levels are 2- to 50-fold higher than levels in adjacent normal intestinal mucosa, while COX-1 levels are unchanged (Kojima *et al.*, 2001; Stack and Dubois, 2001). Although the mechanism of action of PUFA is still unclear, the identification of an enzyme COX-2 catalysing fatty acid oxidation as a rate limiting step in the progress from normal cell growth through hyperplasia on to neoplasia has opened up a new field of research (Reddy, 2000; Bakhle, 2001). This enzyme is a participant in the pathway of colon carcinogenesis, especially when mutation of the Adenomatous Polyposis Coli tumour suppressor gene is the initiating event (Fosslien, 2000). It seems that there is a correlation between COX-2 expression and the size of the tumours and their propensity to invade underlying tissues (Emery *et al.*, 2001; Prescott and Fitzpatrick, 2000). DHA down-regulates the expression of COX-2 and induces apoptosis (Albino *et al.*, 2001). Inhibition of COX activity, decreases eicosanoid production and prevents lung cancer in animal models (Casibang and Moody, 2002). There is a report that feeding menhaden oil in place of corn oil or EPA in place of linoleic acid decreases the development of 1,2- dimethyl hydrazine- or it's metabolite azoxymethane-induced colon tumors, but adding menhaden oil to a low fat diet does not affect colon carcinogenesis (Reddy and Maruyama, 1986). Thus, consumption of diet enriched in n-3 PUFA, specifically EPA and DHA provide a significant mechanism for the prevention of human cancers. Some conflicting results are also there regarding the ability of PUFA in preventing cancers.

Adding fish oil to a diet containing adequate poly unsaturated fatty acids enhances azaserine- induced carcinogenesis in rats and N- nitrosobis (2 oxopropyl) amine-induced carcinogenesis in hamsters (Appel and Woutersen, 1995; 1996). A meta-analysis of experimental animal studies found that n-6 fatty acids strongly enhanced carcinogenesis, mono unsaturated fatty acids had no effect, and n-3 fatty acids weakly (but non-significantly) inhibited carcinogenesis (Fay *et al.*, 1997). It is possible that oxidation products of poly

unsaturated fatty acids could act on signal transduction pathways leading to altered cell proliferation or apoptosis (Welsch, 1992). In addition, lipid peroxidation products can form DNA adducts such as 8-hydroxyguanosine (Beckman and Ames, 1997) which have the potential to exert genotoxicity and therefore could bring about tumor initiation.

PUFA and Liver Disease

Liver disease must be one of the major causes of PUFA deficiency because long chain PUFA biosynthesis mostly occurs in the liver. PUFAs are synthesized from their essential precursors in the smooth endoplasmic reticulum, especially in the liver, by successive desaturation (i.e., oxidation with double bond formation) and elongation (i.e., lengthening of the chain with two methylene groups) reactions. PUFA deficiency is a well-established feature of advanced cirrhosis mainly in plasma, erythrocytes and platelets (Owen *et al.*, 1982; Wilcox *et al.*, 1978). Despite never being measured, the activity of liver desaturases is probably decreased in human cirrhosis, mainly because of liver insufficiency which explains PUFA deficiency in cirrhosis (Cabre and Gassull, 1996). PUFA deficiency may decrease the fluidity of cell membranes and hence impair their biological functions. Decrease in fluidity has been reported either in red blood cells (Owen *et al.*, 1982) or hepatocytes (Schuller *et al.*, 1986) of patients with cirrhosis as compared with healthy controls. Arachidonate deficiency may lead to impaired platelet aggregation often occurring in advanced cirrhosis (Cabre and Gassull, 1996). It has been reported that changes in membrane lipid composition hamper the insulin receptor function in the erythrocytes of cirrhotic patients (Peterson *et al.*, 1992) and that the infusion of poly unsaturated lecithin improves such a derangement (Cantafora *et al.*, 1992). Eicosapentaenoic acid (EPA; 20:5n-3) up-regulates the metabolic action of insulin and inhibits cell proliferation (Murata *et al.*, 2001). It has been found that fish-oil rich in EPA inhibit DEN-induced hepatocarcinogenesis in rats (Sasagawa *et al.*, 2002). On the other hand, some experimental studies have reported that, in alcohol fed rats, a PUFA enriched diet leads to more severe liver injury than a diet enriched in saturated fatty acids (Nanji *et al.*, 1989; Nanji *et al.*, 1995).

An explicative hypothesis proposed is that PUFA increase lipid peroxidation (Piquet *et al.*, 2004). It is suggested that while giving PUFA supplements to cirrhotic patients balance between n-6 and n-3 long chain PUFAs should be ensured as administering only latter (as fish oil) might further impair the already deranged platelet aggregation of these patients (Cabre and Gassull, 1996). As the cirrhotic patients are deficient in antioxidant vitamins (Moscarella *et al.*, 1994) providing the same along with PUFA may create some beneficial effects. Poly unsaturated fatty acids deficiency is common in patients with alcoholic liver disease. Piquet *et al.* (2004) described it as an adaptive mechanism. They found that PUFA deficiency reverses alcohol-related mitochondrial dysfunction *via*. an increase in phospholipid arachidonic over linoleic ratio, which raises cytochrome oxidase activity.

PUFA and Aging

PUFA deficiency is related to a number of diseases like Alzheimer's disease, Liver cirrhosis,

Parkinson's disease, hypertension, cancer, diabetes, inflammatory and auto-immune disorders, depression, schizophrenia, multiple sclerosis etc. Indeed, deficits in the peripheral amounts of PUFA have been described in subjects suffering from neurological and psychiatric disorders. n-3 PUFA deficiency is found to elevate and n-3 PUFA enrichment is found to reduce the brain 2-2-Arachidonoyl glycerol level in mice (Watanabe *et al.*, 2003). 2-Arachidonoyl glycerol is a putative endogenous ligand for cannabinoid receptors and was suggested to play an important role in both physiological and pathological events in the central nervous system (CNS) as well as in peripheral organs. Studies conducted in pregnant rats by Armitage *et al.* (2003) showed that inadequate levels of DHA in the perinatal period are associated with altered blood pressure control in later life.

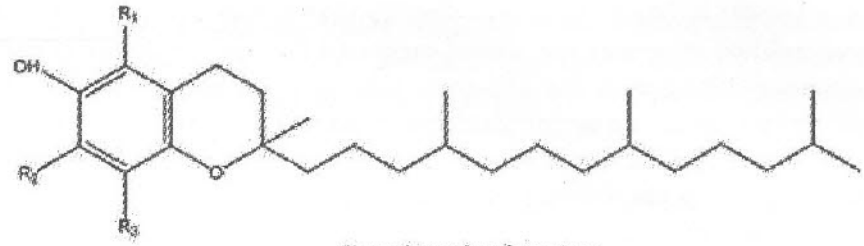
Pepe (2000) observed a distinct decrease in the ratio of mitochondrial membrane ω-3 to ω-6 poly unsaturated fatty acids (PUFA) and a decrease in the mitochondrial phospholipid cardiolipin in aged rat hearts. It has been found that rat cardiomyocytes are devoid of the ability to convert PUFA C20 into C22, although the reverse reaction precedes rather effectively (Mohammed *et al.*, 1990). Moreover, EPA and DHA biosynthesis in animal and human organisms is rather a slow process which is further decelerated with ageing (Chautan *et al.*, 1990).

From birth to aging the heart undergoes functional changes which reflect biochemical and ultrastructural modifications. Indeed, while in the fetus the right ventricle is the dominant pumping chamber, after birth a functional left ventricular dominance develops, following a post-natal increase in systemic vascular resistance and a decrease in pulmonary vascular resistance (Smolich, 1995). Yet cardiac output at rest and during exercise is similar in both young and old healthy subjects. Diastolic function decreases with age, since left ventricular filling is fast in young subjects and slow in the old ones and is due, respectively, to rapid and slow ventricular relaxation and atrial contraction (Roffe, 1998). These functional changes are paralleled by modifications in the number of myocytes (smaller and more abundant in neonatal than in adult and aged heart), in the number of specialized conduction cells (Schluter and Piper, 1999), in the development of cardiac fibrosis due to changes in the amount and composition of connective tissue, in the reduction in calcium transport across membranes, in the lower capillary density, and in the changes in mitochondrial function (Miquel *et al.*, 1992; Lesnefsky *et al.*, 2001). In both humans and animals, the agingprocess in the heart has been associated with a decrease in the total number of myocytes, mainly confined to left ventricle and reactive hypertrophy of the remainingcells (Anversa *et al.*, 1986; Anversa *et al.*, 1990). This cell loss occurs with aging even in the absence of pathologies known to cause heart damage, such as atherosclerosis, diabetes, hypertension, and ischemic heart disease. Apoptosis and necrosis are the two major factors contributing to the loss of cardiac myocytes with age. The study by Kajstura *et al.* (1996) in which apoptosis in the left ventricles was more than 200% in 24-month-old male Fischer rats when compared with 16-month-old rats, strongly suggests that apoptosis may bemore prevalent than necrosis in very old rats.

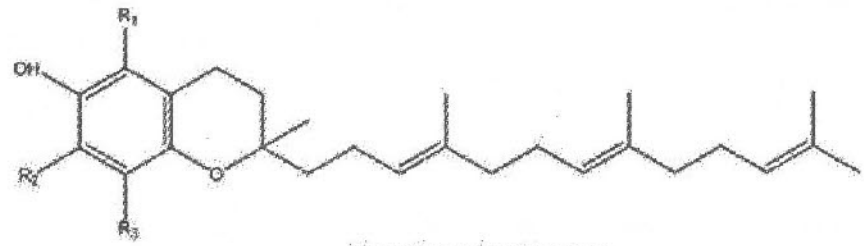
Oxidative stress is one of the major factors that induce apoptosis (Phaneuf and Leeuwenburgh, 2002). Pamplona *et al.* (2000) suggested that the low degree of tissue fatty acid unsaturation of longevous homeothermic animals could have been selected during evolution to protect the tissues against oxidative damage. The heart is one of the organs in which the effects of oxidative damage would be more readily detectable due to its high dependence on oxidative phosphorylation to derive energy. In particular, the heartmay be especially susceptible to mitochondrial dysfunction due to myocardial dependency on ß-oxidation of fatty acids for energy and the post-mitotic nature of cardiac myocytes, which would allow for greater accumulation of mitochondrial mutation sand deletions (Hagen *et al.*, 2002). It is generally agreed that isolated mitochondrial preparations from old compared to young hearts produce more reactive oxygen species (ROS), reflecting an age-related decline incoupling of electron transport to ATP production (Suh *et al.*, 2001). Despite an increasein manganese superoxide dismutase and selenium-dependent glutathione peroxidase activities, there was an increase in lipid peroxidation in the cytosol in myocytes of the old animals compared with the young andmiddle-age groups (Phaneuf and Leeuwenburgh, 2002). Lower oxygen consumption by myocytes (which acts as a compensatory mechanism to the mitochondrial oxidant flux) of 28 month old rats compared to 2 month old rats along with loss of ascorbate suggests that decline in antioxidant status may be a significant contributing factor in the apparent age-related increase in myocardial oxidative stress (Suh *et al.*, 2001).

Vitamin E

Vitamin E is a vitamin that dissolves in fat. It is found in many foods including fish oils, vegetable oils, cereals, meat, poultry, eggs, fruits, vegetables, and wheat germ oil. It is also available as a supplement. Vitamin E, the most active form is alpha-tocopherol, widely distributed in nature with different biological activities. It is a major lipid-soluble antioxidant responsible for protecting membranes against lipid peroxidation which could slow the aging process in humans or animals. Several roles of vitamin E have been reported such as antioxidant, intermediary in arachidonic acid and prostaglandin metabolism, nucleic acid, protein and lipid metabolism, mitochondrial function, sex hormones production, in maintaining the integrity of membranes, protection against hemolytic anemia and impaired erythropoiesis, reducing the risks of heart disease, cancer, neurological diseases, cataract, retinopathy of premature infants and arthritis. Vitamin E deficiency results in neurological syndrome in people with chronic mal-absorption. It is useful in the neurological diseases such as Parkinson's, Huntington's, epilepsy and Tardiv dyskinesia. Several clinical applications of vitamin E are known in diseases such as abetalipoproteinemia, cystic fibrosis, cholestic liver disease, hemolytic anemias, respiratory distress, epilepsy, burns, aging, cancer, ischemic heart disease and cataract. The future study of vitamin E in humans or animal models should provide more definitive evidence of its absorption, transport, utilization and retention in various body organs and tissues as well as in protection and prevention of major neurological diseases.



General tocopherol structure



General tocotrienol structure

α-tocopherol/tocotrienol: R1= CH3, R2= CH3, R3= CH3
β-tocopherol/tocotrienol: R1=CH3, R2= CH3, R3=CH3
γ-tocopherol/tocotrienol: R1=H, R2= CH3, R3=CH3
δ-tocopherol/tocotrienol: R1=H, R2=H, R3=CH3

Fig 6. Structure of Vitamin E

The role of vitamin E as the body's primary lipid-soluble antioxidant (Wang and Quinn, 1999) makes it a good candidate for investigation of effects against diseases of aging, especially those that involve ROS as a main component. Vitamin E is a lipid soluble group of compounds,which includes the stereoisomers a-tocopherol, b-tocopherol, g-tocopherol, and d-tocopherol, along with the 4 tocotrienols-a, b, g, and d-tocotrienols (Fig. 6). While most studies on vitamin E are performed with α-tocopherol, it is important to note that the other tocopherol and tocotrienol stereoisomers and their metabolites appear to have potent biological properties.

Many of these properties appear to be independent of the antioxidant functions of vitamin E. For example, α-tocopherol has been shown to influence cell signaling by inhibiting protein kinase C (PKC) (Azzi *et al.*, 1997). Gene regulatory functions have also been reported for vitamin E as the tocopherols have been shown to potentially serve as ligands for the transcription factor peroxisome proliferator-activated receptor-g (PPAR-g) (De Pascale *et al.*, 2006). Evidence for these roles of the vitamin have been reviewed thoroughly by Traber and Atkinson (2007). Among their conclusions was that the primary role of vitamin E is as a lipid peroxyl radical scavenger *in vivo* and this role likely mediates the effects vitamin E has on cell signaling pathways.

Vitamin E is used for treating vitamin E deficiency, which is rare, but can occur in people with certain genetic disorders and in very low-weight premature infants. Some people use vitamin E for treating and preventing diseases of the heart and blood vessels including hardening of the arteries, heart attack, chest pain, leg pain due to blocked arteries, and high blood pressure. Vitamin E is also used for treating diabetes and its complications. It is used for preventing cancer, particularly lung and oral cancer in smokers; colorectal cancer and polyps; and gastric, prostate, and pancreatic cancer.

Some people use vitamin E for diseases of the brain and nervous system including Alzheimer's disease and other dementias, Parkinson's disease, night cramps, restless leg syndrome, and for epilepsy, along with other medications. Vitamin E is also used for Huntington's chorea, and other disorders involving nerves and muscles. Women use vitamin E for preventing complications in late pregnancy due to high blood pressure (pre-eclampsia), premenstrual syndrome, painful periods, menopausal syndrome, hot flashes associated with breast cancer, and breast cysts.

Taking 200 IU of vitamin E by mouth for more than 10 years seems to help prevent death from bladder cancer. Vitamin E might slow down the worsening of memory loss in people with moderately severe Alzheimer's disease. But vitamin E does not seem to prevent moving from mild memory problems to full-blown Alzheimer's disease. Taking vitamin E for two days before and for three days after bleeding begins seems to decrease pain severity and duration, and reduce menstrual blood loss. Taking vitamin E by mouth seems to reduce anxiety, craving, and depression in some women with premenstrual syndrome. Taking vitamin E before and after treatment with cisplatin chemotherapy might reduce the chance of getting nerve damage.

Some research shows that taking vitamin E might slightly decrease the chance of having a stroke caused by a blood clot (ischemic stroke). But taking vitamin E might also increase the chance of having a more severe type of stroke, called hemorrhagic stroke. This kind of stroke occurs when there is bleeding into the brain.

In liver disease called nonalcoholic steatohepatitis, taking 400-1200 IU vitamin E daily seems to significantly improve symptoms in adults and children after 4-24 months of treatment. Natural vitamin E (RRR-alpha-tocopherol) can significantly improve symptoms in people with early Huntington's disease, but it doesn't seem to help people with more advanced disease.

Taking vitamin E by mouth in combination with vitamin C, beta-carotene and zinc might slow the worsening of advanced age-related macular degeneration (AMD). There isn't enough information to know if this combination helps people with less advanced macular disease or prevents AMD. Zinc needs to be present in the combination for any effect on AMD.

Vitamin E taken along with standard treatment is better than standard treatment alone for reducing pain in people with Rheumatoid arthritis (RA). But this combination doesn't

reduce swelling (inflammation). Sometimes vitamin E is used to lessen the harmful effects of medical treatments such as dialysis and radiation. It is also used to reduce unwanted side effects of drugs such as hair loss in people taking doxorubicin and lung damage in people taking amiodarone. Vitamin E is sometimes used for improving physical endurance, increasing energy, reducing muscle damage after exercise, and improving muscle strength.

Vitamin E is also used for cataracts, asthma, respiratory infections, skin disorders, aging skin, sunburns, cystic fibrosis, infertility, impotence, chronic fatigue syndrome (CFS), peptic ulcers, for certain inherited diseases and to prevent allergies. Some people apply vitamin E to their skin to keep it from aging and to protect against the skin effects of chemicals used for cancer therapy (chemotherapy).

There is inconsistent evidence about the role of vitamin E in asthma. Some research suggests that getting more vitamin E from the diet seems to prevent asthma. But getting vitamin E from supplements doesn't have the same benefit. Some research suggests a combination of vitamin E with vitamin C, beta-carotene, selenium, and zinc which lowers the risk of cancer in men, but not in women. Researchers suspect that men get a lower amount of these vitamins from food, so they might benefit more from supplements. Taking vitamin E plus beta-carotene or vitamin C and beta-carotene does not seem to prevent stomach cancer. But there is limited evidence that getting more vitamin E from the diet might slow the progress of stomach cancer. Taking vitamin E with aged garlic extract and vitamin C might be useful for sickle cell anemia. There is some evidence that synthetic vitamin E (all-rac-alpha-tocopherol) might help prevent stroke in male smokers who have high blood pressure and diabetes.

Vitamin A

Vitamin A is essential for good vision, a healthy immune system, and cell growth. There are two types of vitamin A. This entry is primarily about the active form of vitamin A - retinoids - that come from animal products. Beta-carotene is among the second type of vitamin A, which comes from plants. High doses of antioxidants (including vitamin A) may actually do more harm than good. Vitamin A supplementation alone, or in combination with other antioxidants, is associated with an increased risk of mortality from all causes, according to an analysis of multiple studies. Topical and oral retinoids are common prescription treatments for acne and other skin conditions, including wrinkles. Oral vitamin A is also used as a treatment for measles and dry eye in people with low levels of vitamin A. Vitamin A is also used for a specific type of leukemia.

Vitamin A has also been studied as a treatment for many other conditions, including cancers, cataracts, and HIV. However, the results are inconclusive. Most people get enough vitamin A from their diets. However, a doctor might suggest vitamin A supplements to people who have vitamin A deficiencies. People most likely to have vitamin A deficiency are those with diseases (such as digestive disorders) or very poor diets. Seafood is a very good food source of retinoid vitamin A. Other animal sources rich in vitamin A are eggs, whole

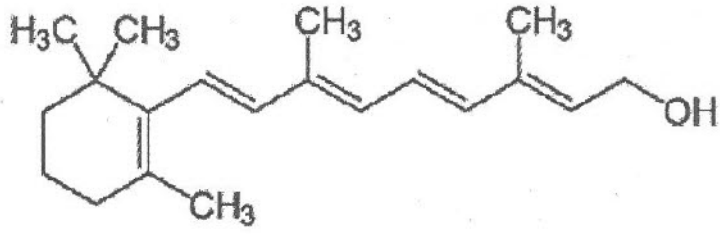


Fig 7. Structure of Vitamin A

milk, liver, fortified skim milk. Plant sources of vitamin A (from beta-carotene) include carrots, spinach, and apricots.

Vitamin K

Vitamin K plays a key role in helping the blood clot, preventing excessive bleeding. Unlike many other vitamins, vitamin K is not typically used as a dietary supplement. Vitamin K is actually a group of compounds. The most important of these compounds appears to be vitamin K_1 and vitamin K_2. Vitamin K_1 is obtained from leafy greens and some other vegetables. Vitamin K_2 is a group of compounds largely obtained from seafood, meats, cheeses, and eggs, and synthesized by bacteria. Recently, some have looked to vitamin K to treat osteoporosis and steroid-induced bone loss.

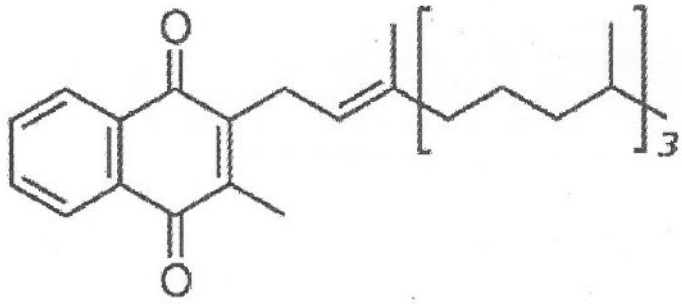


Fig. 8. Structure of Vitamin K_1

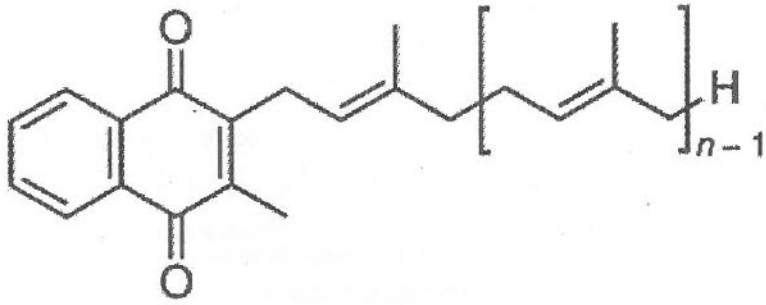


Fig. 9. Structure of Vitamin K_2

Low levels of vitamin K can raise the risk of uncontrolled bleeding. While vitamin K deficiencies are rare in adults, they are very common in newborn infants. A single injection of vitamin K for newborns is standard. Vitamin K is also used to counteract an overdose of the blood thinner Coumadin. While vitamin K deficiencies are uncommon, you may be at higher risk if you:

- Have a disease that affects absorption in the digestive tract, such as Crohn's disease or colitis
- Take drugs that interfere with vitamin K absorption
- Are severely malnourished
- Drink alcohol heavily

Uses of vitamin K include the one for cancer, for the symptoms of morning sickness, for the removal of spider veins, and for other conditions.

Carotenoids

Carotenoids are chemicals that have nutritive properties and that exist in the pigment that colours plants and animals. As fat-soluble materials, carotenoids are ingested by humans in countless colourful fruits, vegetables and marine fishes. They are important as antioxidants and for their capacity to get converted to essential vitamins. Marine animals contain various carotenoids that show structural diversity. These marine animals accumulate carotenoids from foods such as algae and other animals and modify them through metabolic reactions. Many of the carotenoids present in marine animals are metabolites of β-carotene,

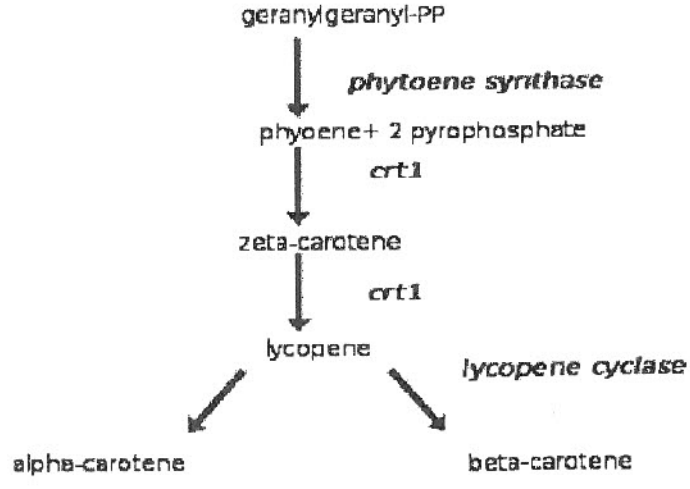


Fig. 10. Biosynthesis of carotenoids

fucoxanthin, peridinin, diatoxanthin, alloxanthin, and astaxanthin. Carotenoids found in these animals provide the food chain as well as metabolic pathways. Among the 750 reported carotenoids found in nature, more than 250 are of marine origin. In particular, allenic carotenoids, except for neoxanthin and its derivatives, and all acetylenic carotenoids originate from marine algae and animals.

In general, animals do not synthesize carotenoids *de novo*, and so those found in animals are either directly accumulated from food or partly modified through metabolic reactions. The major metabolic conversions of carotenoids found in animals are oxidation, reduction, translation of double bonds, oxidative cleavage of double bonds, and cleavage of epoxy bonds. Carotenoids have been traditionally used in food and animal feed due to their colour properties. The natural carotenoids are used to reinforce fish colour, which increases consumers' perception of quality. An example is the addition of carotenoids to fish feed to impart colour to farmed salmon. The nutraceutical properties of carotenoids also attracted attention of the food industry. Large numbers of scientific studies have confirmed the benefits of carotenoids to health and use for this purpose is growing rapidly. Besides, carotenoids have been proposed as value-added compounds that could contribute to make microalgal biofuel production economically feasible. The main applications of carotenoids are currently as dietary supplements, fortified foods, food colour, animal feed and pharmaceuticals and cosmetics.

β-carotene, the most widely known of the carotenoids, is known to be a vitamin A precursor, similar to several other carotenoids. Carotenoids have antioxidant properties and a large number of studies have confirmed their benefits to health. In particular, carotenoids are thought to reduce the risk of degenerative diseases and cancer especially in elderly people.The health industry uses carotenoids in over-the-counter (OTC) dietary supplements and fortified foods. This is one of the fastest growing segments of the industry but is still relatively small compared to the colour segment. The pharmaceutical and cosmetics industries also use carotenoids mainly for their colouring properties, though their use by the pharmaceutical and cosmetics companies is growing rapidly due to their nutraceutical properties. An example of a new product from this segment is a 'beauty pill' containing the carotenoid lycopene. This product belongs to a new market segment known as 'cosmeceuticals', which aims to combine cosmetics and nutraceutical food ingredients to create products to improve skin and hair.

When acting as antioxidants, carotenoids have been shown to reduce the damage caused by certain molecules called free radicals. A generous amount of these chemicals might prevent damage to cells and tissues as well as genetic damage. This means that they might increase a person's immunity to infection, reduce the risk of cancer and protect against heart disease. The potential benefits of beta-carotene are believed to include preventing hardening of the arteries, which is associated with an increased risk of heart attack. Beta-carotene also preserves the health of the body's mucous membranes and lining of the lungs, which are among the body's first lines of defense against infection. Excessive amounts of beta-carotene,

however, might bring a higher risk of osteoporosis or of lung cancer in smokers.

Carotenoids might help to fight serious infections in people who have compromised immunity systems by boosting their white blood cell count. Lycopene has been associated with a reduced incidence of several types of cancer, including prostate cancer, colon cancer, bladder cancer and lung cancer. Zeaxanthin and lutein are believed to strengthen the eyes, protecting against cataracts and macular degeneration. Although some of these connections have yet to be clearly established through scientific studies and research, health experts typically recommend that people should consume the recommended daily amounts of fruits and vegetables to get the full benefits of carotenoids.

Marine animals (shrimp, lobster and salmon) are rich in carotenoids. Carotenoids are also responsible for the colours of some birds (flamingo, canary), certain insects, and marine animals (shrimp, lobster and salmon). Carotenoids are important factors in human health and essential for vision. The role of beta-carotene and other carotenoids as the main dietary source of vitamin A has been known for the better part of this century. More recently, protective effects of carotenoids against serious disorders such as cancer, heart disease and degenerative eye disease have been recognized, and have stimulated intensive research into the role of carotenoids as antioxidants and as regulators of the immune response system.

Carotenoid prevents oxidation of low density lipoprotein (LDL) cholesterol and reduces the risk of developing atherosclerosis and coronary heart disease. High LDL oxidation is associated with increased risk of atherosclerosis and coronary heart disease. Carotenoid content in the body can be achieved by increasing the consumption of seafood. The bound chemical form of carotenoids is converted by the temperature changes involved in processing to make it more easily absorbed by the body. Ongoing research suggests that carotenoids can reduce the risk of prostate cancer and cancers of the lung, bladder, cervix and skin.

Carotenoids are not essential in the nutritional sense. However, they are beneficial for animal health. It is well-known that carotenoids have an unsubstituted β-end group, such as β-carotene, α-carotene, and the β-cryptoxanthin precursor of vitamin A in animals. Furthermore, canthaxanthin was also converted to retinol in Salmoidae fish. 3-Hydroxy carotenoids: lutein, zeaxanthin, and astaxanthin, were also reported to be precursors of 3,4-dehydroretinol (Vitamin A_2) in some freshwater fish. Many marine animals accumulate carotenoids in their integuments. Integumentary carotenoids may contribute to photoprotection, camouflage, and signaling such as breeding colour. Carotenoids have excellent antioxidative activities for quenching singlet oxygen and inhibiting lipid peroxidation. Astaxanthin supplementation in Salmonidae fish suppresses oxidative stress. Marine animals also accumulate carotenoids in their gonads. Carotenoids are assumed to be essential for reproduction in marine animals. Astaxanthin supplementation in cultured salmon and red sea bream increases ovary development, fertilization, hatching, and larval growth. In the case of the sea urchin, supplementation with β-carotene, which was metabolized to echinenone, also increased reproduction and the survival of larvae.

Carotenoids also enhance immune activity in marine animals. Carotenoids are used for pigmentation in several aquaculture fish. Synthetic and natural astaxanthin from Phaffia yeast and Haematococcus algae is widely used for the pigmentation of salmon, trout, and red sea bream. Lutein from marigold is also used as a yellow colouration for cultured marine fish such as yellow tail and red sea bream. Zeaxanthin from spirulina is used as a red colouration for goldfish and ornamental carp.

Pro-vitamin A function

One of the most important functions of carotenoids in the human body is their ability to convert into retinol (provitamin A function), a faculty that about 10% of carotenoids identified in nature possess. Vitamin A is well recognized as a factor of great importance for child health and survival, its deficiency causes disturbances in vision and various related lung, trachea and oral cavity pathologies. Animals and humans cannot synthesize carotenoids *de novo*, although they are able to convert them into vitamin A. Diet is the only source for these precursors for retinol synthesis, fruits, vegetables and microalgae being the major suppliers of provitamin A active carotenoids. As a reference value, a recommended daily intake of 6 mg of carotenoids has been proposed. This value is based on the contribution of compounds with provitamin A activity, specially β-carotene, which has been assigned a provitamin A activity of 100%.

Carotenoids and Cancer

In recent years, epidemiological evidence supporting a protective effect of carotenoids to the development of chronic and degenerative diseases has grown considerably. We must not forget that cancer and cardiovascular diseases are the leading causes of death in the world and that approximately 50% of all tumors are attributed to the diet.From a nutritional point of view, an antioxidant can be defined as any substance present in foods that significantly reduces the adverse effects of reactive oxygen species in normal physiological conditions in humans. Antioxidants, in particular carotenoids, are essential for cell health due to their protective action on cellular components against oxidative damage. These activities have generated two lines of research related to the physiological functionality of carotenoids: on one hand, their activity as membrane antioxidants, therefore involved in the oxidative cell cycle and, on the other hand, their involvement in control processes of cell differentiation and proliferation. As an example of the first, a recent study showed that antioxidant enzymes including catalase, superoxide dismutase and peroxidase levels in plasma and liver of mice increased significantly when the animals were fed with microalgae biomass (*Haematococcus pluvialis*, *Scenedesmus platensis*or *Botryococcus braunii*), which reveals an increased antioxidant protection against free radicals.

Carotenoids would directly act on DNA in order to regulate the production of RNA that is responsible for 'gap-junctions' communications, which could successfully explain some anti-tumor activities of carotenoids. Immune system cells also require intercellular communication to conduct their activity efficiently, so the previous action mechanism of

carotenoids could also apply for supporting the immune system activity. As an example, high doses of β-carotene increase the CD4 to CD8 lymphocyte ratio, which is very low in patients suffering from HIV disease. In the last decades, many laboratory and epidemiological studies have been conducted which suggest that intake of carotenoids and cancer prevalence are inversely related. Among the carotenoids, lycopene has been one of the most extensively studied, probably due to the greater anticancer capacity shown with respect to other carotenoids.

Carotenoids and Cardiovascular Diseases

Cardiovascular diseases are the leading cause of death in developed countries, and have become the main health problem also in developing countries. These include acute myocardial infarction and disorder of high morbidity and mortality. Oxidative stress and inflammation are the main factors contributing to the pathophysiology of these disorders. In particular, the oxidative stress induced by ROS can cause low density lipoproteins oxidation (LDL), an aspect that plays a key role in the pathogenesis of atherosclerosis.

Another major feature of carotenoids is protection of LDL against oxidation, which confers carotenoids anti-atherogenic properties. In addition, carotenoids have been shown to inhibit *in vivo* lipid peroxidation processes, by which the presence of carotenoids in cell membranes is essential to act as stabilizing elements of these structures. In this sense, the antioxidant activity of some carotenoids during radical peroxide-induced cholesterol oxidation was investigated by Palozza *et al.*, showing that carotenoids exerted a significant antioxidant activity, in the decreasing activity order indicated: astaxanthin, cantaxanthin, lutein and β–carotene. Several researchers have published that daily dietary α-carotene supplementation in mammals led to decreased plasma levels of total lipids, cholesterol and triglycerides.

Numerous epidemiological studies suggest that diets rich in carotenoids could protect the human body from certain cardiovascular diseases due to the involvement of oxidizing substances and oxidative stress in the development and clinical expression of coronary heart disease. Low α-carotene levels in serum have been shown to inversely correlate prevalence of coronary artery disease and formation of arterial plaque, by which α-carotene has been proposed as a potential marker for human atherosclerosis. In addition, carotenoids displaying high levels of provitamin A activity, including α-carotene, β-carotene and β-cryptoxanthin, have been associated with reduced risk of angina pectoris disease. Other epidemiological studies have also found low levels of oxygenated carotenoids (namely xanthophylls: lutein, zeaxanthin, lycopene, β-cryptoxanthin, β-carotene and α-carotene) in plasma of patients with acute and chronic coronary syndromes. High levels of β-cryptoxanthin and lutein in plasma have been shown to decrease risk for suffering from myocardial infarction.

Carotenoids and Eye Health

Many research studies showed that lutein and zeaxanthin are the main responsible

pigments for both the yellowing and the maintenance of normal visual function of the human eye macula, while other major carotenoids in serum (α-carotene, β-carotene, lycopene and β-cryptoxanthin), are absent or are found in trace amounts in the human macula. In the eye macula, lutein and zeaxanthin absorb blue light and also attenuate pernicious photooxidative effects caused by the excess blue light, while reducing eye chromatic aberration. Due to their antioxidant properties, carotenoids protect the eye macula from adverse photochemical reactions. Major prevalency of cataracts has also been linked to people with low levels of lutein and zeaxanthin. Also macular degeneration, the main cause of irreversible loss of vision in people above 65 years in industrialized countries, has been associated with very low levels of lutein and zeaxanthin.

The spectra of lutein and zeaxanthin show a wide absorption band with a peak at 450 nm, which is thought to be involved in absorbing excess blue light before it comes to photoreceptors, therefore preventing the eye macula from being damaged by blue light. Moreover, due to lutein's and zeaxanthin's biophysical and biochemical properties for ROS scavenging, these carotenoids might also preserve the membrane structure in the eye photoreceptors from lipid peroxidation processes, in contrast to non-polar carotenoids as lycopene and β-carotene. Concentration of lutein and zeaxanthin in the retina can be increased on diet bases (spinach and maize) and on supplements of both pigments.

Other Physiological Functions of Carotenoids

Carotenoids provide skin photoprotection against UV light. Due to their scavenging action on ROS, carotenoids also possess anti-inflammatory properties. Astaxanthin raises anti-inflammatory effects while preserving essential lipids and proteins of human lymphocytes. Astaxanthin would act by inducing superoxide dismutase and catalase enzyme activities. Astaxanthin protects from hepatic damage by inhibiting lipid peroxidation, stimulating the cellular antioxidant system and modulating the inflammatory process. Carotenoids have been used as preservatives in cosmetics and, combined with other antioxidants or algal bioactive substances, also in creams and lotions for sun protection. The beneficial effect of carotenoids has also been shown in patients with psoriasis, skin inflammatory pathology. Lima and Low levels of carotenoids in the skin correlate well with psoriasis prevalence. Finally, it is interesting to note that, in recent years, carotenoids are being considered as important protective molecules in gastric disorders. It has been published that a high intake of carotenoids prevents the development of disorders caused by *Helicobacter pylori*, a Gram negative bacteria genus that colonizes the gastric mucosa of at least half of the human population.

Carotenoids have many physiological functions. Carotenoids are efficient free-radical scavengers, and they enhance the vertebrate immune system. There are several dozen carotenoids in foods people consume, and most carotenoids have antioxidant activity.Epidemiological studies have shown that people with high β-carotene intake and high plasma levels of β-carotene have a significantly reduced risk of lung cancer. However,

studies of supplementation with large doses of β-carotene in smokers have shown an increase in cancer risk (possibly because excessive β-carotene results in breakdown products that reduce plasma vitamin A and worsen the lung cell proliferation induced by smoke. Similar results have been found in other animals. Not all carotenoids are helpful, e.g. etretinate is a teratogen.

Humans and animals are mostly incapable of synthesizing carotenoids, and must obtain them through their diet. The notable exception is the red pea aphid, which has the genes necessary for synthesizing carotenoids, thought to have been acquired from fungi via horizontal gene transfer. Carotenoids are a common and often ornamental feature in animals. For example, the pink colour of flamingos and salmon, and the red colouring of cooked lobsters are due to carotenoids. It has been proposed that carotenoids are used in ornamental traits (for extreme examples see puffin birds) because, given their physiological and chemical properties, they can be used as honest indicators of individual health, and hence they can be used by animals when selecting potential mates.

In the macula lutea of the human eye, certain carotenoids are actively concentrated to the point that they cause a yellow colouring, and this may help to protect the retina from blue and actinic light, in the same way that carotenoids protect the photosystems of plants. Carotenoids are also actively concentrated in the corpus luteum of the ovaries, where they impart the characteristic colour, and may act as general antioxidants.

Ether lipids

Scandinavian folk medicine used shark liver oil for the treatment of cancers and other ailments based on the rarity of tumors in sharks and their ability to resist infections. Shark liver oil is a source of alkyl glycerols which have been studied as anti-cancer agents in several clinical trials. Moreover, alkyl glycerols have been investigated for the treatment of radiation induced side effects and for their ability to boost the immune system. Several experimental studies have shown the ability of alkyl glycerols to open the blood brain barrier to facilitate the access of therapeutic drugs to the central nervous system. Shark liver oil contains both alkylglycerols (AKG) and squalene and is an ancient remedy among the fishermen along the west coast of Norway and Sweden. It has been used for wound healing, the treatment of irritations of the respiratory and alimentary tracts and lymphadenopathy. Structurally they are alkyl ethers of glycerol (Figure 11).

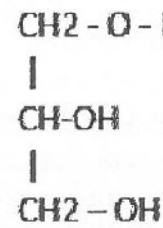


Fig. 11. Chemical structure of alkylglycerols (AKG)

Alkyl glycerols and Immunity

AKG and alkyl lysophospholipids significantly activate cytotoxic macrophages leading to enhanced Fc-receptor mediated phagocytosis and increase humoral immune response and delayed hypersensitivity reaction. AKG have been shown to stimulate hematopoiesis, erythropoiesis, thrombocytosis and granulocytosis in animals.

Alkyl glycerols and Cancer

Hajimoradi *et al.* investigated ether lipid activity as an immunomodulator. They used peritoneal macrophages from female inbred BALB/c mice, aged 8 to 10 weeks, to determine the *in vitro* effect of 100% pure natural arctic shark liver oil, extracted from the Greenland Shark *Somniosus microcephalus* and observed that, with the increase of shark liver oil concentration, nitric oxide (NO) production of macrophages increased with no multiplication of cells thus supporting the use of shark liver oil as an adjunct therapy in the treatment of neoplastic disorders and as an immune booster in infectious diseases. Apte *et al.* described the preparation of pure 1-*O*-hexadecyl-2-palmitoyl-*sn*-glycero-3-phospho-(*N*-palmitoyl) ethanolamine as well as of the corresponding 1-*O*-tetradecyl, 1-*O*-octadecyl-, and 1-*O*[(*Z*)-9'-octadecenyl]-derivatives, each of which exhibits a strong antitumor effect in cultured neoplastic cells and in mice bearing MC 11 sarcomas.

Pedrono *et al.* observed that orally AKG, administered to mice affected by Lewis lung carcinoma tumors, reduced metastasis dissemination by 64 ± 8%, whereas SLO effect was 30 ± 9% below control. Purified AKG also decreased significantly plasmalogen content in tumors, whereas shark liver oil had no such effect. A 5-day treatment with AKG curtailed the presence in tumors of von Willebrand factor, a marker of endothelial cells suggesting an anti-angiogenic effect of AKG. This study shows that AKG decrease the growth, vascularization, and dissemination of carcinoma tumors in mice.

The anticancer effect of AKG might be due to the property of activating macrophages, and increasing the cytokines such as IL-12 and IFN-gamma production. The recruitment and activation of macrophages, in fact, are acknowledged to be fundamental in the primary antitumor defense while IFN-gamma, a T-cell derived lymphokine, inhibits a wide number of malignant cells, either directly counteracting cancer cells growth or through its immunomodulatory properties.

Alkyl glycerols and blood brain barrier

Intracarotid short-chain AKG constitute a very effective and low toxic strategy for transient opening of the BBB to overcome the limited access of cytotoxic drugs to the brain. Short-chain AKG shows to increase the transfer of MTX to the brain and several instruments have been described to control the AKG mediated increase in barrier permeability. *In vitro* and *in vivo* assessment reveal that this new concept of BBB opening is associated with no toxic effects at therapeutic levels. Therefore intra-carotid AKG represent a new therapeutic procedure to overcome the limited access of therapeutic agents to the CNS. Erdlenbruch

et al. investigated a method to chemically open the BBB using intra-arterial administration of AKG to increase the transfer of erucyl phosphocholine (ErPC), a new derivative of the alkyl phosphocholine (APC) family, into the brain. In contrast to hexadecylphosphocholine, the prototypical APC, ErPC can be administered intravenously without causing hemolysis. ErPC possesses a cell membrane-mediated mechanism of action which is thought to be responsible for its ability to overcome the chemoresistance against standard anticancer agents frequently observed in high-grade gliomas.

Alkyl glycerols and Radiation Therapy

Inhibition of tumor growth and a decrease of the number of both radiation and complex injuries were observed when AKG had been administered prior to radiation treatment of patients affected by cancer of the uterine cervix. In particular this study highlights that prophylactic administration of AKG markedly reduced fistulas proving the potential clinical importance of AKG as a complement to conventional radiation therapy in cancer treatment.

In conclusion, marine organisms are potential sources of variety of lipids and lipophilic compounds with varied biomedical and pharmaceutical applications. Yet little has been explored in this aspect. Further research has to be carried out to utilize the marine lipids for the welfare of mankind.