FISH OIL: NUTRACEUTICAL AND BIOMEDICAL APPLICATIONS

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Fish oil is the primary natural long chain omega-3 fatty acid source containing two human health beneficial fatty acids. They are rich in polyunsaturated 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 (nonessential 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 polyunsaturated 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.1)** and docosahexaenoic acid (DHA, C22:6n-3) (**Fig 2**). 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.

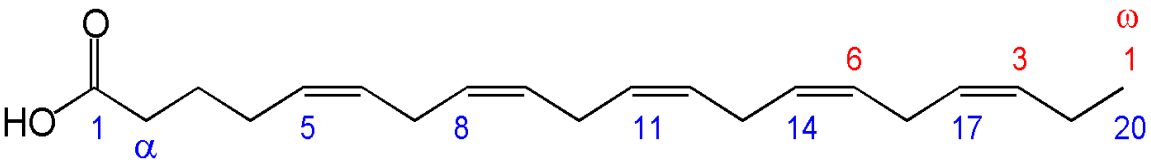

Fig 1 Chemical structure of eicosapentaenoic acid (EPA)

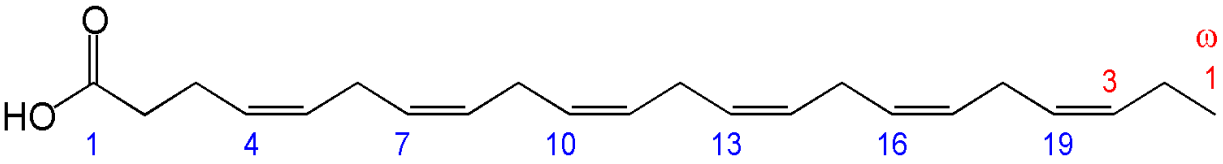

Fig 2.3.1.2 Chemical structure of docosahexaenoic acid (DHA)

Metabolism

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 6^{th} 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. 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.

Nutritional and health benefits of Fish oil and PUFA consumption

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 postprandial lipemia (Harris *et al.,* 1988). Chylomicron remnants are selectively cleared after the ingestion of n-3 PUFA. 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. 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.

PUFA and lipid profile

Monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid-rich diets decrease the levels of total plasma cholesterol and LDL-cholesterol and increase HDL-cholesterol in healthy normolipidemic subjects 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. 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 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 and in disorders such as psoriasis. 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). Studies 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 polyunsaturated content of the myocardial membranes would be expected to influence their susceptibility to lipid peroxidation. 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 times 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. The low occurrence of fatal coronary heart disease in Eskimos could be related to their high intake of marine n−3 PUFA (10-14g/day). This ecological study was the basis for the hypothesis that consumption of marine n−3 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. 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).

The mortality following myocardial infarction was reduced by 29% after 2 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. 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, phosphatidylethanolamines and phosphatidylcholines) which constitute up to 90% of the overall phospholipids pool of the myocardial membranes. 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. In neonatal cardiomyocytes cells, arrhythmia caused by agents such as high extra cellular calcium, ouabain, isoproterenol or lysophosphatidylcholine was prevented by exogenous EPA in the free form. As removal of free EPA with added bovine serum albumin quickly reversed this protective effect, 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. 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. Interestingly, exogenous DHA supplementation has also been demonstrated to correct calcium homeostasis and mitochondrial dysfunction in diabetic cardiomyocytes. As inhibition of protein kinase C is associated with a reduction in reactive oxygen species generation, DHA has been shown to inhibit generation of superoxide from neutrophils. 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

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. 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. In humans, dietary (n-3) fatty acid treatment offers possibilities in malignant diseases. 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. In patients with prostate cancer, fish intake was inversely related to cancer. 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. Although the mechanism of action of PUFA is still unclear, the identification of an enzyme COX-2 catalyzing 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. 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. It seems that there is a correlation between COX-2 expression and the size of the tumours and their propensity to invade underlying tissues. DHA down-regulates the expression of COX-2 and induces apoptosis. Inhibition of COX activity, decreases eicosanoid production and prevents lung cancer in animal models. 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. 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 polyunsaturated fatty acids enhances azaserine-induced carcinogenesis in rats and N- nitrosobis (2 oxopropyl) amine- induced carcinogenesis in hamsters. A meta-analysis of experimental animal studies found that n-6 fatty acids strongly enhanced carcinogenesis, monounsaturated fatty acids had no effect, and n-3 fatty acids weakly (but nonsignificantly) inhibited carcinogenesis. It is possible that oxidation products of polyunsaturated fatty acids could act on signal transduction pathways leading to altered cell proliferation or apoptosis. In addition, lipid peroxidation products can form DNA adducts such as 8-hydroxyguanosine (Beckman & Ame 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. 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. 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 or hepatocytes of patients with cirrhosis as compared with healthy controls. Arachidonate deficiency may lead to impaired platelet aggregation often occurring in advanced cirrhosis. It has been reported that changes in membrane lipid composition hamper the insulin receptor function in the erythrocytes of cirrhotic patients and that the infusion of polyunsaturated lecithin improves such a derangement. Eicosapentaenoic acid (EPA; 20:5n-3) up-regulates the metabolic action of insulin and inhibits cell proliferation. It has been found that fish-oil rich in EPA inhibit DEN-induced hepatocarcinogenesis in rats. 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.

An explicative hypothesis proposed is that PUFA increase lipid peroxidation. 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. As the cirrhotic patients are deficient in antioxidant vitamins providing the same along with PUFA may create some beneficial effects. Polyunsaturated fatty acids deficiency is common in patients with alcoholic liver disease. 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-Arachidonoylglycerol level in mice. 2-Arachidonoylglycerol 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 rat dams 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.

A distinct decrease in the ratio of mitochondrial membrane ω-3 to ω-6 polyunsaturated 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. Moreover, EPA and DHA biosynthesis in animal and human organisms is rather a slow process which is further decelerated with ageing.

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 postnatal increase in systemic vascular resistance and a decrease in pulmonary vascular resistance. 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. 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, 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. In both humans and animals, the aging process 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 remaining cells. 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.

Oxidative stress is one of the major factors that induce apoptosis (Phaneuf & Leeuwenburgh, 2002)**.** 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. 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 in coupling of electron transport to ATP production. Despite an increase in 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 and middle-age groups (Phaneuf & Leeuwenburgh, 2002)**. 7.**

PUFA and Neuroprotection

In the fetal brain, DHA, a structural constituent of membranes accumulates mainly during the last trimester of pregnancy and remains at very high proportions up to the end of the second year of life. Consumption of DHA may contribute to optimal conditions for brain development as the endogenous formation of DHA appears to be pretty low. Researches reveal that DHA is essential for brain function mainly for neuronal cell growth, differentiation as well as in neuronal signaling. (Lauritzen et al., 2016). Triglyceride form of DHA facilitates neuroprotection in experimental Parkinson's disease, a neurodegenerative disorder and many studies suggest that omega-3 polyunsaturated fatty acids provides protection against brain damage. (Gómez-Soler et al., 2018).

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