FISH OIL

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Dietary sources and biosynthesis pathways represents the main suppliers for the fatty acids needed by the human body to construct hydrophobic parts of Bio molecules. Various animal and vegetable lipids are ingested, hydrolyzed at least partially by digestive enzymes and absorbed through the intestinal wall to be distributed through the body , first in the lymphatic system and then in the blood stream . To some extent such dietary supply may adjust the composition of fatty acids in the body lipids (1).

Metabolic processes in the tissues of the normal human body can modify both dietary fatty acids additionally that are synthesized in these tissues, to produce various structures that are needed. The actual composition of the fatty acids supplied in the diet is relatively unimportant since an appropriate proportions of the relatively highly unsaturated fatty acids are requested. Actually many higher mammals, including humans are unable to produce fatty acids with double bonds very far towards the end of the molecule either during de novo synthesis or by modification of the dietary acids. Accordingly these fatty acids can provides essential fatty acids and acting also as carriers for oil soluble vitamins like A,D,E ,K additionally their intestinal absorption . The oils derived from cold-water fish are unique among all the edible oils and fats which represents an important source of long chain ω-3 or n-3 polyunsaturates (2).

Fatty acids group referred to as conjugated Linoleic acid are receiving now greater interest due to having health promoting properties . The liver oils from fish such as cod and halibut have an added features as very important natural source for oil soluble vitamins like A and D (3).

Two forms of polyunsaturates are considered essential, known as n-3 and n-6 where average intake of the former have fallen considerably as compared to the second . Accordingly an adequate intake of performed long chain ω-3 polyunsaturated fatty acids from fish must be occured. Dietary 18 c fatty acids (essential fatty acids) was previously referred to as vitamins F1 and F2. These 18 c fatty acids (Linoleic and α linolenic) are converted by a common enzyme system into 20, 22 carbon derivatives. Since the conversion enzymes are the same for both n-3 and

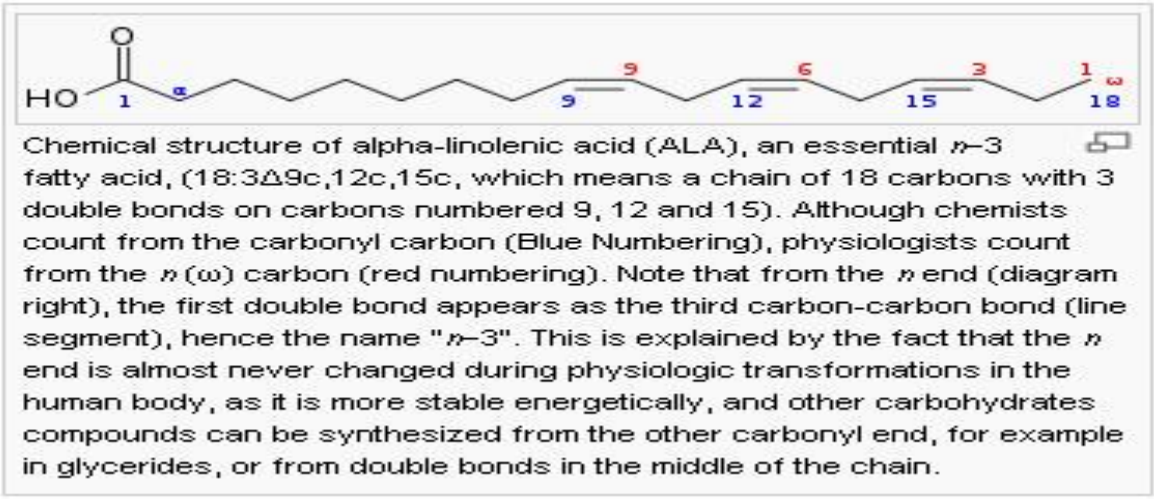
n-6 the phenomenon of competitive inhibition occurs. Accordingly the predominance of one family may deviate the other away from enzyme sites and the conversion process favor the first one. As a result an excess of the dietary ω-6 will inhibit ω-3 conversion to 20, 22 carbons from specifically eicosapentaenoic and Docosahexenoic acids (4).

n−3 fatty acids (popularly referred to as ω−3 fatty acids or omega-3 fatty acids) are members of a series of essential unsaturated fatty acids that have in common a final carbon–carbon double bond in the n-3 position; that is, the third bond from the methyl end of the fatty acid (5).

Nutritionally important n−3 fatty acids include α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), all of which are polyunsaturated. The human body cannot synthesize n−3 fatty acids de novo, but it has a limited ability to form the "long chain" n−3 fatty acids EPA (20 carbon atoms) and DHA (22 carbon atoms) from the "short chain" eighteen-carbon n−3 fatty acid ALA (6).

Essential fatty acids are molecules that cannot be synthesized by the human body but are vital for normal metabolism. One of the two families of these essential fatty acids are the omega-3 fatty acids. Because omega (ω) is the last letter in the Greek alphabet the naming system counts from the last of the carbons to the carbon-carbon double bond. Similarly, the preferred n–3 nomenclature uses the letter "n" to mean the number of carbon atoms in the chain. If the fatty acid has this third-to-last double bond, it is called an ω–3 ("omega minus 3") fatty acid. Common sources of n–3 fatty acids include fish oils and some plant oils such as flaxseed oil (7).

Chemical structure of alpha-linolenic acid (ALA), an essential n−3 fatty acid, (18:3Δ9c,12c,15c, which means a chain of 18 carbons with 3 double bonds on carbons numbered 9, 12 and 15). Although chemists count from the carbonyl carbon (Blue Numbering), physiologists count from the n (ω) carbon (red numbering). Note that from the n end (diagram below), the first double bond appears as the third carbon-carbon bond (line segment), hence the name "n−3". This is explained by the fact that the n end is almost never changed during physiologic transformations in the human body, as it is more stable energetically, and other carbohydrates compounds can be synthesized from the other carbonyl end, for example in glycerides, or from double bonds in the middle of the chain (8).



Chemical structure of alpha-linolenic acid (ALA), an essential *n*−3 fatty acid, (18:3Δ9c,12c,15c, which means a chain of 18 carbons with 3 double bonds on carbons numbered 9, 12 and 15). Although chemists count from the carbonyl carbon (Blue Numbering), physiologists count from the *n* (ω) carbon (red numbering). Note that from the *n* end (diagram right), the first double bond appears as the third carbon-carbon bond (line segment), hence the name "*n*−3". This is explained by the fact that the *n* end is almost never changed during physiologic transformations in the human body, as it is more stable energetically, and other carbohydrates compounds can be synthesized from the other carbonyl end, for example in glycerides, or from double bonds in the middle of the chain.

Chemical structure of eicosapentaenoic acid (EPA).

Chemical structure of docosahexaenoic acid (DHA).

The term n−3 (also called ω−3 or omega-3) signifies that the first double bond exists as the third carbon-carbon bond from the terminal methyl end (n) of the carbon chain. n−3 fatty acids which are important in human nutrition are: α-linolenic acid (18:3, n−3; ALA), eicosapentaenoic acid (20:5, n−3; EPA), and docosahexaenoic acid (22:6, n−3; DHA). These three polyunsaturates have either 3, 5 or 6 double bonds in a carbon chain of 18, 20 or 22 carbon atoms, respectively. All double bonds are in the cis-configuration; in other words, the two hydrogen atoms are on the same side of the double bond. Most naturally-produced fatty acids (created or transformed in animal or plant cells with an even number of carbon in chains) are in cis-configuration. Like free oxygen radicals, iodine can add to double bonds of docosahexaenoic acid and arachidonic acid forming iodolipids (9).

List of *n*−3 fatty acids

This table lists several different names for the most common *n*−3 fatty acids found in nature.

Common name	Lipid name	Chemical name
Hexadecatrienoic acid (HTA)	16:3 (*n*−3)	*all-cis*-7,10,13-hexadecatrienoic acid
α-Linolenic acid (ALA)	18:3 (*n*−3)	*all-cis*-9,12,15-octadecatrienoic acid
Stearidonic acid (SDA)	18:4 (*n*−3)	*all-cis*-6,9,12,15-octadecatetraenoic acid
Eicosatrienoic acid (ETE)	20:3 (*n*−3)	*all-cis*-11,14,17-eicosatrienoic acid
Eicosatetraenoic acid (ETA)	20:4 (*n*−3)	*all-cis*-8,11,14,17-eicosatetraenoic acid
Eicosapentaenoic acid (EPA)	20:5 (*n*−3)	*all-cis*-5,8,11,14,17-eicosapentaenoic acid
Heneicosapentaenoic acid (HPA)	21:5 (*n*−3)	*all-cis*-6,9,12,15,18-heneicosapentaenoic acid
Docosapentaenoic acid (DPA), Clupanodonic acid	22:5 (*n*−3)	*all-cis*-7,10,13,16,19-docosapentaenoic acid
Docosahexaenoic acid (DHA)	22:6 (*n*−3)	*all-cis*-4,7,10,13,16,19-docosahexaenoic acid
Tetracosapentaenoic acid	24:5 (*n*−3)	*all-cis*-9,12,15,18,21-tetracosapentaenoic acid
Tetracosahexaenoic acid (Nisinic acid)	24:6 (*n*−3)	*all-cis*-6,9,12,15,18,21-tetracosahexaenoic acid

A typical fish oil (softgel)is that derived from the tissues of oily fish. Fish oils contain the omega-3 fatty acids eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), precursors of eicosanoids that are known to reduce inflammation throughout the body and are thought to have many health benefits (10).

Fish do not actually produce omega-3 fatty acids, but instead accumulate them from either consuming microalgae that produce these fatty acids, as is the case with fish like herring and sardines, or, as is the case with fatty predatory fish, by eating prey fish that have accumulated omega-3 fatty acids from microalgae. Such fatty predatory fish like shark, sword fish, tilefish, and albacore tuna may be high in omega-3 fatty acids, but due to their position at the top of the food chain, these species can accumulate

toxic substances. For this reason, the FDA recommends limiting consumption of certain (predatory) fish species (e.g. albacore tuna, shark, and swordfish) due to high levels of toxic contaminants such as mercury, dioxin, PCBs and chlordane (11).

Fish oil is used as a component in aquaculture feed. More than 50 percent of the world's fish oil used in aquaculture feed is fed to farmed salmon. Fish oil has been studied for treating clinical depression, anxiety, and enhancing the benefits from depression medications. Countries with the highest intake of fish in their diets are correlated with the lowest rates of depression among citizens (12&13).

Fish oil Production

In 2005, fish oil production declined in all main producing countries with the exception of Iceland. The 2005 production estimate is about 570,000 tonnes in the five main exporting countries (Peru, Denmark, Chile, Iceland and Norway), a 12% decline from the 650,000 tonnes produced in 2004. Peru continues to be the main fish oil producer worldwide, with about one fourth of total fish oil production. Though Peruvian catches of fish, intended to be reduced in 2005, were more or less in line with the 2004 result, fish oil production declined from 350,000 tonnes to 290,000 tonnes, due to lower fat content of the fish. In the recent summer months, the fat content was as low as 2% which compares to 4% in 2004. Despite an 18% decline in production, Peruvian earnings from fish oil exports reached US$156 million in 2005, exceeding the 2004 income by US$6 million. This was due to an increase in fish oil prices (14).

Production process Description: Fish processing includes both Fish canning for human consumption and the production of fish bi products such as meal and oil .Canning itself includes Two methods:

A- Precooking method

B- Raw back method

A) Precooking: The raw fishes are cleaned, cooked before the canning step. It is used typically for larger fish such as Tuna.

B) Raw back: The raw fishes are cleaned, placed in cans before cooking ,used for small fish such as Sardines.

The byproduct manufacturing segment of the fish industry uses canning or filleting wastes, fish that are not suitable for human consumption to produce fish meal and fish oil (15).

Caning:

A) Precooking method begins with :

1- Thawing of the fish, eviscerated, washed, cooked using steam oil, hot air or smoke for 1.5- 10 hours depending on the fish size. Such step removes the fish oils, coagulates the protein in the fish to loosen the meat and then cooled for several hours. It may be refrigerated to reduce the time.

2- Head, fins, bones and undesirable meat are removed, the remainder is cut or chooped and placed in cans. Oil, brine and or water are added to the cans which are sealed and pressure cooked before shipment.

B) Raw back method

1- It begins with thawing , weighing the fish , washed , possibly brined or nobbed which removes the head ,viscera and tail

2- The Fish are placed in cans, cooked, drained and dried.

3- After drying liquid (oil, brine, water, sauce or other) is added to the cans, then sealed, washed and sterilized with steam or hot water (16).

Fish byproduct manufacturing :

1- It begins with cooking the fish at 100◦ c (lower for some species) in a continuous cooker. This process coagulates the protein and rupture the cell walls to release water and oil.

2- The mixture may be strained with an auger in a perforated casing before pressing with a screw press.

3- As the fishes are moved along the screw press , the pressure is increased and the volume is decreased.

4- The liquid from the mixture (pressing liquor) is squeezed out through a perforated casing .

5- The pressing liquor which consists of water, oil and some solids is transported to a centrifuge to remove solids.

6- These solids are later returned to the press cake in the drying step .

7- The oil and water are separated using a disc –type centrifuge in the oil separator .

8- The oil is polished by using hot water washes, centrifuged ,then sent to an oil – refining operation , the water is removed from the oil (stick water) goes to an evaporation to concentrate the solids (17).

The press cake , stick water , solids are mixed and sent to either a direct-fired or indirect-fired dryer (steam tube dryer).
A direct- fired dryer consists of a slowly rotating cylinder through which air is heated to about 600 ◦ C by an open flame , passes through the mishes to evaporate the liquid (17).
The indirect one is a fixed cylinder with rotating crapers that heat the meal with steam or hot fluids flowing through discs , tubes , coils or the dryer casing itself . Air also passes through the apparatus , but is not heated, flows in the opposite direction to the meal to entrain the evaporated water.
Such process may rquire twice as much time to the direct one procedure .
The dried meal is cooled , ground , stored in bulck or in paper, used in animal and pet feeding owing to its high protein content.

The polished oil

It is further purified by a process called hardening . First the polished oil is refined by mixing the oil with an alkaline solution in a large stirred vessel . The alkaline solution react with free fatty acid in the oil to form insoluble soaps . the mixture is allowed to settle overnight , the cleared oil is extracted off the top , then washed with hot water to remove any remaining soap (18).

Bleaching

This is accomplished by mixing the oil with natural clays to remove oil pigment, colored matter at temperature 80-116 ◦c in either a batch or controlled mode (19).

Hydrogenation

It is the next step where hydrogenation of the unsaturated fatty acids chain. Nickel catalyst at concentration of 0.05-0.1 percent by weight is

added to the oil mixture, heated ,stirred and hydrogen is injected into the mixture to react with unsaturated fatty acids chain . The oil is cooled after complete hydrogenation, filtered to remove the nickel.
The hydrogenated oil is refined again before the de oderization step which removes odor and flavor producing chemicals.
De oderization occur in a vacuum chamber where dry oxygen free steam is bubbled through the oil to remove the undesirable chemicals. Volatilization of the undesirable chemical occur at temperature between 170-230◦C. The oil is then cooled to about 38◦C before exposure to air to prevent formation of undesirable chemicals (20).

Fish oil is obtained by extracting total fish body with food grade ethanol and purified by molecular distillation to remove various contaminants (Fig.1). The crude fish oil preparations contain 90% fatty acid triacylglycerols, 2-5% unsaponifiable matter containing sterols, fatty acid-estrified cholesterol, free fatty acids, minor quantities of fat soluble vitamins. (Figure 1 and Table 1) (21).

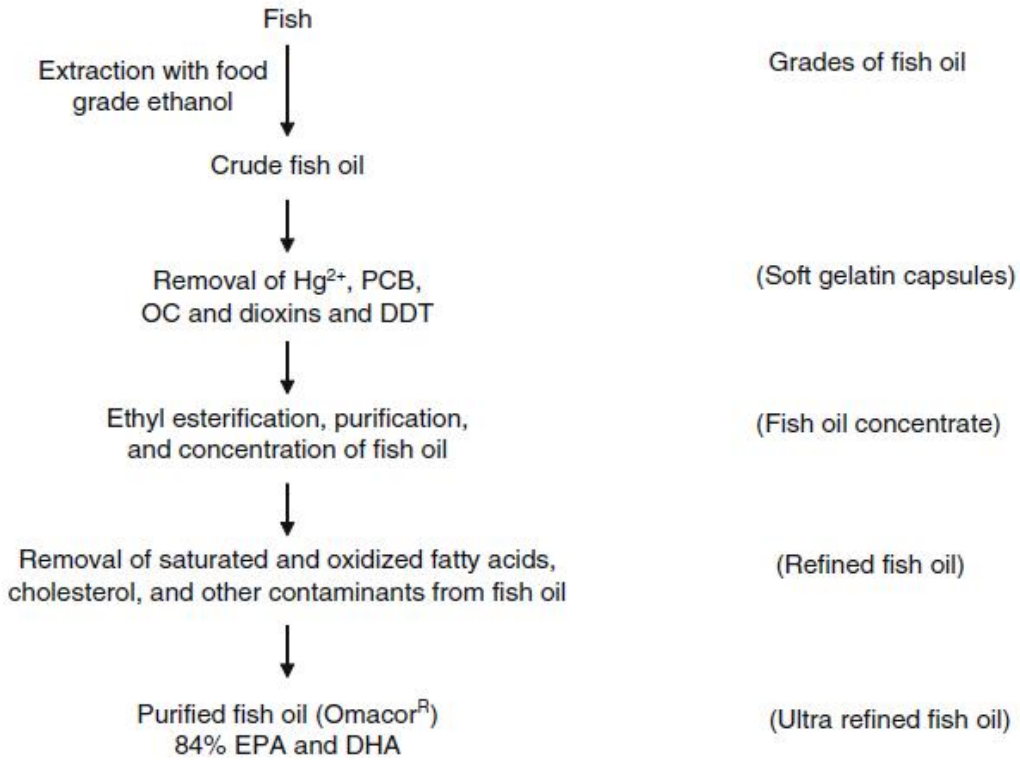


Figure 1. Procedure for the purification of ultrapure fish oil from crude fish oil by molecular distillation.

Table 1. Commercial names and manufacturers of n–3 fatty acid (DHA and EPA) preparations from fish and algal sources

Commercial name	Manufacturer
Purified fish oil	Carlson Laboratories Inc, Arlington Heights, IL, USA
Omegaven™	Kabi Bad Homburg, Germany
Lipoplus™ (LMF541)	B. Braun Melsungen, AG
Omacor™	Reliant pharmaceutical Inc Corner, NJ, USA
Zodin®	Solvary, Takeda, Kuhnil Pharmaceutical Co Ltd, Pierre Fabre Sante, France
DHASCO-S and T	Martek Biosciences Corporation, Columbia, MD, USA
Cardiozen™	Equazen Nutraceuticals, London, UK
DHActive TMCL	Nutrinova, Montpellier, France
Neuramin DHA	AllStar Health, Huntington, CA, USA
O-Mega-Zen3™	Nu Tru Inc, Lincolnwood, IL, USA
Lovaza	GlaxoSmithKline, Philadelphia
Efalex™	Scotia Pharmaceuticals, Scotland, UK
Marinol D-40	Loders Croklaan Lipid Nutrition, Wormerveer, The Netherlands
Vegan omega-3	Deva Nutrition, Chelsea, AL, USA
Krill oil	Jedwards International inc, Quincy, MA, USA

Fish oil Consumption

Epidemiologic studies have shown a link between total fat consumption and development of chronic degenerative diseases such as cancer (22). Several studies have reported a positive association between high fat intake and the incidence of breast, colon, and prostate cancer (23).
However n-3 polyunsaturated fatty acids (PUFAs) present in oily fish appear to be protective (FO) (24).

In general the western diet has a ratio of ω-6 to ω-3 fatty acids of about 15:1. The Paleolithic diet on which human beings evolved, and lived for most of their existence, had a ratio of 1:1 and having high content of antioxidants (25). Changes in eating habits, natural versus processed food, and agricultural development within the past 100 to 200 years caused these changes in the ω-6 to ω-3 ratio. The decreased consumption of DHA-enriched foods and increased consumption of ω-6 enriched vegetable oils is responsible for the 15:1 ω-6: ω-3 ratio (26). Western diet therefore promotes the pathogenesis of many chronic diseases such as cardiovascular ,inflammatory, autoimmune and neurodegenerative diseases, whereas a diet enriched in ω-3 fatty acids exerts cardioprotective, immunosuppressive, and neuroprotective effects. A lower AA: DHA ratio suppresses the human disorders that mentioned above. The American Heart Association recommends that everyone should eat oily fish twice per week and that those with coronary heart

disease should consume 1 g/day of EPA plus DHA from oily fish or supplements (27).

Incorporation of ω-3 fatty acids into functional foods is limited by their high susceptibility to oxidative degradation. An oil-in-water emulsion may be a more effective method to deliver ω-3 fatty acids into functional foods (28). The best sources of dietaryω-3 fatty acids are fish and fish oil. Fish oil is available in the form of a purified liquid in lemon flavor (Carlson Laboratories Inc, Arlington Heights, IL), a 10% fish oil emulsion Omegaven™ (Kabi Bad Homburg, Germany), 20% fish oil emulsion Lipoplus™(MLF541) (B. Braun Melsungen AG), and an FDA approved fish oil preparation Omacor™ (Reliant Pharmaceuticals, Inc., Corner, NJ). Omacor is composed of approximately 90% ω-3 fatty acids (465 mg EPA, 375 mg DHA, and > 60mg other ω-3 fatty acid esters), for a total of > 900mg of ω-3 fatty acids per 1 g capsule. Cardiozen™(Equazen Nutraceuticals, London,UK) is another fish oil preparation that contains 6 times more EPA than DHA. These fish oil preparations lower plasma triacylglycerol level, increase bleeding time, downregulate gene expression for platelet-derived growth factor, lower blood pressure, decrease the number of endothelial adhesion molecules, improve lipoprotein size, and decrease the risk of cardiovascular and cerebrovascular diseases. Fish oil is well tolerated and has no significantly adverse effects. In neural membranes, EPA and DHA not only affect their physicochemical properties such asmembrane fluidity, permeability, and viscosity, especially in neuronal synapses, but also modulate neurotransmission (29), gene expression (30), activities of enzymes andreceptors, ion channels, and immunity (31).

According to the American Heart Association (AHA), as well as various international organizations, omega-3 fatty acids benefit the heart of healthy people, and those at high risk or suffering cardiovascular disease (CD). In 1996, the AHA released its Science Advisory, “Fish Consumption, Fish Oil, Lipids and Coronary Heart Disease” (Stone, 1996). Since then, important new findings about the benefits of omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosohexaenoic acid (DHA) have been reported. The current AHA's dietary guidelines recommend that healthy adults eat at least two servings of fish (particularly fatty fish) per week. The AHA suggests that patients without or with documented CD eat, respectively, a variety of fish (preferably oily) at least twice a week, or to consume about 1 g of EPA+DHA per day, preferably from oily fish. However, the Scientific Statement of the AHA also indicates that fish and seafood is potentially a major source of human exposure to various environmental contaminants. Depending on

their stage of life, consumers need also to be aware of the potential health risks of eating fish (32).

Although fish consumption can provide substantial amounts of omega-3 fatty acids however not everyone can gain these important fatty acids, and this is especially true for children (33). Therefore the interest in the development of other foods, such as dairy products enriched with fish oil has increased in recent years (34). Such 'functional foods' could be an effective way to reduce risk factors for several 'lifestyle-related' diseases including CVD, without the need for modification of the consumer's dietary habits in either adulthood or childhood (35).

Consumption of fish oil may protect against cardiovascular disease (36),additionally its intake is inversely associated with fatal coronary heart disease (37) where randomized controlled trials of dietary fish or fish oil supplementation after myocardial infarction demonstrate a reduction in mortality (38). These effects are believed to be due to the high oil contents of omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA must be obtained from dietary sources and recent guidelines from the American Heart Association recommend the consumption of oily fish at least twice weekly for individuals with and without coronary heart disease (39).
Knowledge about differences in consumer perceptions of health risks and benefits related to fish consumption is important for the development of targeted health interventions associated with dietary choice.
Regular fish consumption is part of a healthy diet (40). However, actual fish consumption levels are often far below dietary advice recommending consumption of two portions per week (41,42 ,43).

Fish consumption is associated with both nutritional benefits and toxicological risks to human health. For example,omega three fatty acids in fatty fish can substantially reduce the risk of cardiovascular disease (44), and fatty fish is an important source of vitamin D compared to other food products, which can improve the development of bones (45).

Dietary intervention studies suggest that a daily fish meal can improve blood pressure (BP) which represents one of many causal factor to cardiovascular disease(46) however, such a dietary regimen might be difficult to sustain. Diet plays an important role in the etiology of hypertension, because obesity and nutrient intake are known to affect BP.

The BP-lowering properties of ω-3 long-chain polyunsaturated fatty acids (ω-3 LC-PUFA) from fish oil supplements have been thoroughly

investigated and confirmed (47), with commonly used doses of 4–5 g per day leading to clinically significant reductions in BP. The largest effects are achieved in elderly hypertensives (48). Several biological mechanisms have been suggested in addition regarding its health benefits on BP, e.g., changes in phospholipid composition, platelet aggregation, and vasodilatation (49).

Without the use of seafood supplements (fish oil or marine algae), a ω-3 LC-PUFAs intake of 4–5 g/d can hardly be achieved, even if one consumes a daily fish meal. There are relatively few dietary intervention trials that have investigated the effects of daily fish consumption (in contrast to fish oil ingestion) on BP, and recorded good achievments (50,51)

Fish Oil Supplements and safety

Most commercially available fish oils are derived from coldwater fish, primarily menhaden, but also salmon and trout. These oils are rich in the Omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These two fatty acids are metabolized to series 3 prostaglandins, which have a significant moderating influence on inflammation. Fish, in turn, obtain EPA and DHA from algae, making super green foods such as marine micro-algae , an alternate source of EPA and DHA. Algae may become the dominant source of omega-3 fatty acids as fish populations continue to decline in the world's oceans. Fish oil is readily absorbed as a dietary supplement. After one week of supplementation, increases in omega-3 fatty acids are detectable in the tissues. Tissue elevations in EPA and DHA persist for as long as one to two months after supplementation is discontinued (52).

From a conventional medical perspective, some animals will tend to develop oily coats and large flakes of dander following omega-3 fatty acid supplementation. This condition, known as seborrhoea oleosa, resolves within one to two weeks of discontinuing the supplement. Omega-3 EFAs may inhibit platelet aggregation . This may account for some clinicians to recommend not using them when other anticoagulant (anti-clotting) medications are used. Fish oil neither seem to cause bleeding problems nor increase blood sugar level when it is taken by itself at commonly recommended dosages in contrary to earlier reports(53).

Fish oil health benefits

Fish oil n-3 fatty acid DHA, EPA are essential polyunsaturated fatty acids for the brain since brain tissues lacks sufficient enzymatic activity, necessary for de novo n-3 fatty acid synthesis. They are obtained either directly from the diet or synthesized from its main dietary n-3 precursor in the liver Both types of fatty acids are incorporated as structural components of neural membranes glycerophospholipids and are substrates for generating lipid mediators. From 3-5 percent esterified brain Arachidonic acid and 2-8 percent of esterified rat brain glycerophospholipid DHA are replaced daily with unsaturated fatty acids derived from the plasma pool. The incorporated and proportion of n-3 and n-6 fatty acids markedly influence neural membrane property such as fluidity , permeability and gene expression (54).

The metabolic utilization of n-3 fatty acids differs from that of n-6 metabolism . oxygenation of n-6 fatty acids generates pro inflammatory mediators such as prostaglandins and leukotrienes whereas n-3 fatty acids generate anti-inflammatory lipid mediators such as resolvins , protectins and neuroprotectins. These short –lived lipid mediators act as local hormones. The present –day western diet has a ratio 0f n-6 to n-3 fatty acids of about 15 : 1 . The Paleolithic diet on which human beings evolved and lived for most of their existence had a ratio of 1: 1 and was high in antioxidants (55).

Changes in eating habits, natural versus processed food and agricultural development within the past 100-200 years caused these changes in n-6 to n-3 ratio. The decreased consumption of n-6 enriched vegetable oils is responsible for the 15:1 n-6 : n-3 ratio. The present western diet promotes the pathogenesis of many chronic diseases such as cardiovascular disease, inflammatory, autoimmune and neurodegenerative diseases whereas a diet enriched in n-3 fatty acids exerts cardio protective, immunosuppressive and neuroprotective effects. A lower AA : DHA ratio suppresses the human disorders mentioned above (56). The American heart Association recommends that everyone should eat oily fish twice per week and that those with coronary heart disease should consume 1 G/day of EPA plus DHA from oily fish or supplements. Incorporation of n-3 fatty acids into functional food is limited by their high susceptibility to oxidative degradation . An oil in water emulsion may be more effective method to deliver n-3 fatty acids into functional foods and the best sources of dietary n-3 fatty acids are fish and fish oil. Fish oil preparations can lower plasma Triacylglycerol (TG) level , increase bleeding time , downregulate gene expression for platelet – derived growth factor , lower blood pressure , decrease the number of endothelial adhesion molecules , improve lipoprotein size and

decrease the risk of cardiovascular and cerebrovascular diseases. Therefore Fish oil is well tolerated and has no significant adverse effects (57).

In neural membranes EPA and DHA not only affect their physicochemical properties such as membrane fluidity, permeability and viscosity , especially in neuronal synapses but also modulate neurotransmission, gene expression, activities of enzymes and receptors.. In non –neural tissues , the dietary uptake of n-3 fatty acids reduces pre-atherogenic cytokines , improves endothelial function , reduces vascular occlusion indeed coronary atherosclerosis . Replacement of ω-6 with ω-3 fatty acids in membrane glycerophospholipids decreases the transcriptional activation of many genes including adhesion molecules , chemoattractant and inflammatory cytokines (58).These genes are associated with the response to inflammatory and pro inflammatory stimuli . The quenching of gene expression of proinflammatory pro-atherogenic genes by n-3 fatty acids has consequences on the extent of leukocyte adhesion to vascular endothelium, early atherogenesis , later stages of plaque development and rupture. This may present Great explanation for the vasculoprotective effects of ω-3 fatty acids (58).

The n–3 fatty acids in fish oil markedly affect human health. To achieve a significant increase in human tissues in vivo, intake of high doses of fish oil is required because bioavailability of n–3 fatty acids from fish oil to neural cells involves not only digestion and transport but also metabolism (59). Furthermore, the efficacy of dietary intervention may also depend upon the health of the individual. For example, patients with gastrointestinal problems may not be able to absorb fish oil supplement. Daily use of n–3 fatty acids (3–5 g/day) by normal individuals may optimize the functions of human heart, brain, lungs, liver, spleen, and kidneys (Fig.2). These fatty acids reduce cardiovascular disease, type 2 diabetes, hypertension, cancer, inflammatory and autoimmune diseases, and neurodegenerative diseases (60). In human nutritional studies, attempts have been made to increase the consumption of n–3 fatty acid enriched food because Western diet is deficient in n–3 fatty acids and has excessive levels of n–6 fatty acids compared with the diet on which human beings evolved and their genetic patterns are established. Excessive levels of n–6 fatty acids and a very high n–6/ n–3 ratio (20 : 1), as are found in today’s Western diets, promote the pathogenesis of inflammatory and autoimmune, and neurodegenerative diseases, whereas increased levels of n–3 fatty acids (a lower n–6/ n–3 ratio) exert suppressive effects and prevent cardiovascular disease, type 2 diabetes,

hypertension, cancer, inflammatory and autoimmune diseases, and neurodegenerative diseases(61).

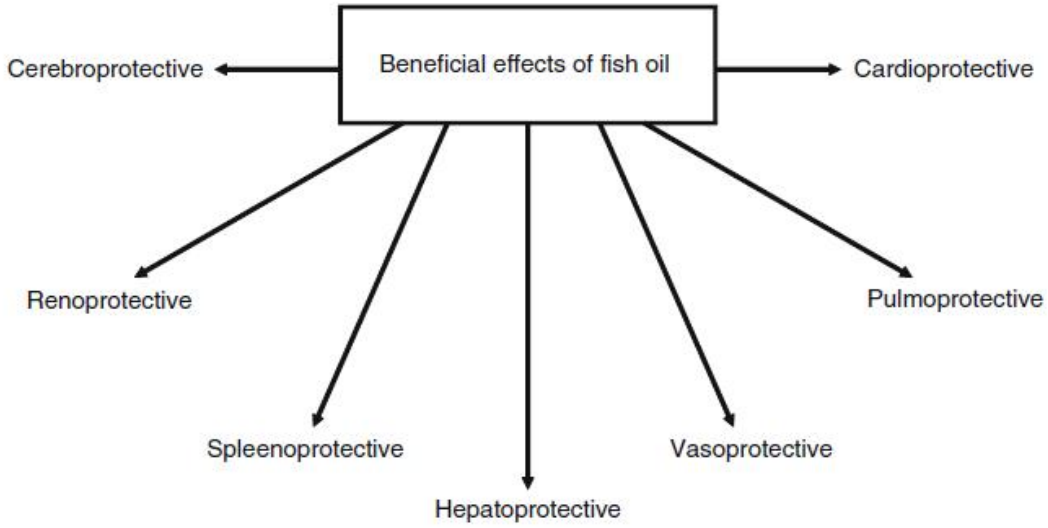


Figure 2. Beneficial effects of fish oil on various organs of human body

Effects of Fish Oil on Heart

In heart, the incorporation of fish oil constituents, n–3 fatty acids, not only influences the physicochemical properties of membranes (fluidity, permeability) but also modulates the expression of many genes (62). The n–3 fatty acids reduce proatherogenic cytokines, improve endothelial function, reduce vascular occlusion, and mitigate the course of coronary atherosclerosis. These fatty acids are also needed for vascular smooth muscle cell function (63). An antiarrhythmic effect is also observed at the supraventricular and ventricular level (64). The n–3 fatty acids also modulate levels of plasma fibrinogen and coagulant factor VII levels (65). Collective evidence suggests that n–3 fatty acids reduce leukocyte reactivity, atherosclerosis, and thrombosis. In addition, fish oil exerts its effect through several different cellular mechanisms including the effects on lipoprotein metabolism, hemostatic function, platelet/vessel wall interactions, antiarrhythmic actions, and also through the inhibition of proliferation of smooth muscle cells and therefore growth of the atherosclerotic plaque.

The majority of cardiovascular benefits of fish oil are mediated in the vascular wall and at the vascular endothelium, which plays a central role in the regulation and maintenance of cardiovascular homeostasis and function (66) through the generation of nitric oxide , eicosanoids in addition to direct effects on contractility.

n–3 fatty acids in fish oil may affect atherogenesis via inhibition of vascular smooth muscle cell proliferation at the gene expression level and

by modifying the expression of inflammatory cytokines and adhesion molecules (67).

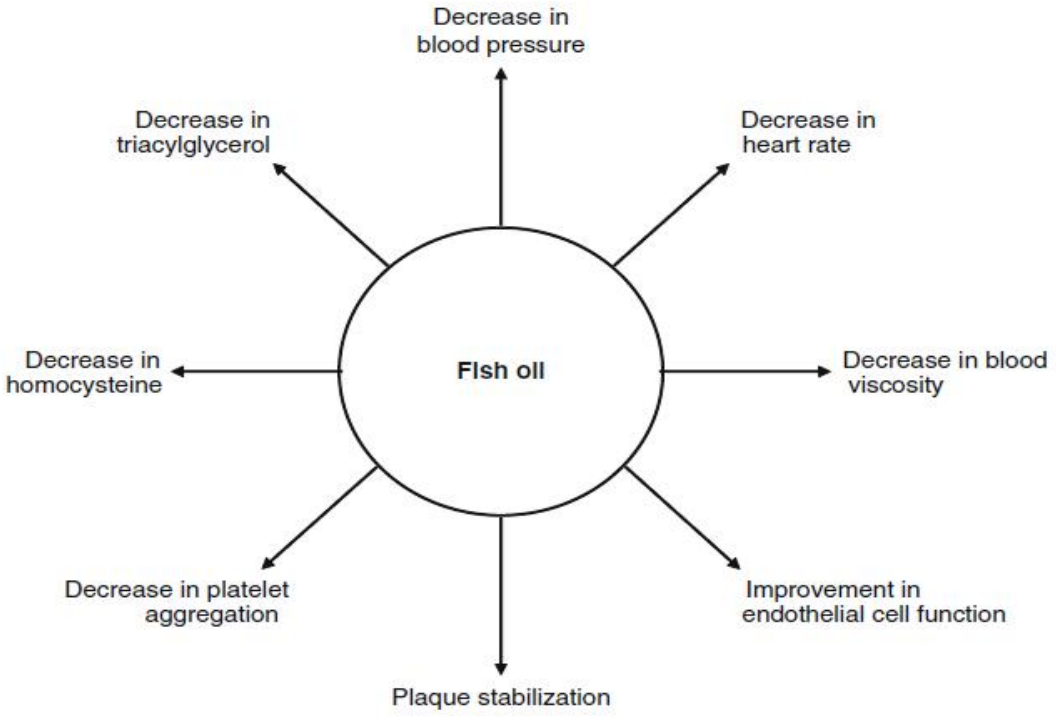


Figure 3. Physiologic and pharmacologic effects of fish oil

Effects of Fish Oil on Brain

The n–3 fatty acids enter brain from circulation across blood–brain barrier and are incorporated at the sn-2 position of glycerol moiety of glycerophospholipids. They are released through the action of phospholipase A2. The high levels of DHA in the retina and brain gray matter suggests that this fatty acid has important roles in retinal and neural function. Animal studies have shown that depletion of DHA from the retina and brain results in reduced visual function and learning deficits (68). Aging not only reduces levels of n–3 fatty acids in brain but also decreases neural membrane neuroplasticity. Decline in n–3 fatty acids with age is accompanied by loss of memory and learning. Reduction in n–3 fatty acid levels with age can be restored by DHA supplementation. Molecular mechanism associated with the loss of n–3 fatty acids from neural membrane is not fully understood. However, it is proposed that the loss of DHA with aging may be due to increased activity of plasmalogen-selective PLA2 (69).

Dietary supplementation of DHA not only restores the levels of DHA but also increases cerebral choline and acetylcholine levels in hypertensive rats and also in rat hippocampus during aging (70).

Effects of Fish Oil on Lungs

Lung diseases such as asthma and bronchitis are characterized by continuous inflammation. Thus, factors and drugs that downregulate inflammatory activity may be particularly beneficial for patients with asthma and bronchitis. Treatment of asthmatic subjects with fish oil or n–3 fatty acid supplements results in improved ARA concentrations in neutrophils, reduced neutrophil chemotaxis, reduced leukotriene generation, and reduced airway late response to allergen exposure. However, relevant data are still sparse (71). Studies in the general adult population indicate that a high fish intake has a beneficial effect on lung function, but the relationship with respiratory symptoms and clinically manifest asthma and bronchitis is less evident (72).

Effects of Fish Oil on Kidneys

Administration of n–3 fatty acids reduces proteinuria and ameliorates renal injury in murine lupus nephritis, experimental focal segmental glomerulosclerosis, and other types of renal diseases (73). Similarly, dietary supplementation of fish oil in dogs produces marked decrease in renal mass, followed by reduction in proteinuria, glomerulosclerosis, tubule interstitial damage, and progressive loss of renal function (74). In contrast, dogs fed on n–6 fatty acid-enriched diet showed progressive deterioration of kidney function associated with proteinuria, hypercholesterolemia, morphologic evidence glomerular , tubule interstitial injury, and increased prevalence of end-stage kidney failure. The molecular mechanism associated with n–6 fatty acid-mediated kidney damage and n–3 fatty acid-induced nephroprotection remains unknown (75).

Arachidonic (ARA) derived lipid mediators are vital for the proper control of renal hemodynamics. The lack of control of ARA-derived mediators can contribute to renal vascular injury and end-stage renal diseases (76). Three major enzymatic pathways, COX (cyclooxygenase), LOX (lipoxygenase), and EPOX (epoxygenase), are responsible for bioactive eicosanoids. These metabolites, through eicosanoid receptor-mediated mechanism, dilate or constrict the renal vasculature and maintain vascular resistance in the face of changing vasoactive hormones. Renal vascular generation of eicosanoids is altered in pathophysiological conditions such as hypertension, diabetes, metabolic syndrome, and acute

renal failure. Collective evidence suggests that n–6-derived vasoactive eicosanoids modulate renal hemodynamics in rats, and early generation of vasoactive eicosanoids may induce glomerular hyperfunction, and subsequent overproduction of thromboxanes is closely associated with the pathogenesis of glomerular damage and proteinuria (77). In contrast, n–3 fatty acids interfere with the ARA cascade not only by competing for COX and LOX enzymes but also by generating eicosanoids that are less potent than ARA-derived eicosanoids. Studies on the effect of EPA on mesangial cells indicate that EPA modulates PDGF-stimulated proliferation by engendering changes in PDGFstimulated TXA2 synthesis. Fish oil also inhibits mesangial cell activation , proliferation, reduces proteinuria, and decreases histologic evidence of glomerular damage (78 ,79).

Effects of Fish Oil on Plasma Lipids

A predominance of small dense low-density lipoprotein (LDL) and increased plasma triacylglycerol levels are increased risk for the development of coronary heart disease. Dietary long-chain n–3 fatty acids modulate the levels of triacylglycerols in several ways. They suppress the synthesis of triacylglycerols in the liver by inhibiting the diacyglycerol acyltransferase activity. However its effects on very low-density lipoprotein (VLDL) synthesis and/or VLDL triacylglycerol removal rate via increased lipoprotein lipolysis have not been ruled out. Fish oil ingredients may limit the supply of lipid substrates for triacylglycerol synthesis. The n–3 fatty acids have been reported to increase the sensitivity of certain tissues to the action of insulin. Thus, in rat liver, synthesis and secretion of triacylglycerol and cholesterol ester are reduced by 50–80% in the presence of EPA in comparison with oleic acid. Reduced synthesis of triacylglycerol and cholesterol ester is seen when EPA and oleic acid are given together. EPA causes higher incorporation of 3H water into glycerophospholipids and lower incorporation into triacylglycerol and cholesterol ester as compared with oleic acid. Reduction in synthesis of triacylglycerol and cholesterol ester is also observed when EPA-CoA is given together with oleoyl-CoA, whereas palmitoyl-CoA, stearoyl-CoA, linolenoyl-CoA, and arachidonoyl-CoA have no inhibitory effects (Rustan and Drevon, 1989). Collectively, these studies suggest that n–3 fatty acid-mediated triacylglycerol-lowering effect and redistributes LDL subfractions toward larger and lighter particles, thus providing protection against the generation of atherogenic type of LDL. This effect is more pronounced in carriers of the apoE4 polymorphism (80).

Furthermore, EPA or EPA-CoA also reduces cellular cholesterol esterification by inhibiting the activity of acyl-CoA:cholesterol acyltransferase. The lowered cholesterol esterification induced by EPA also modulates the secretion of VLDL cholesterol ester. The molecular mechanism associated with hypotriglyceridemic effects of fish oil in humans may include the involvement of liver X receptor, hepatocyte nuclear factor-4a (HNF-4 a), farnesol X receptor, and peroxisome proliferator-activated receptors (PPARs). All these receptors are regulated by sterol receptor element binding protein-1c (SREBP-1c), the main genetic switch that modulates lipogenesis. It is proposed that n–3 fatty acids elicit hypotriglyceridemic effects by coordinately suppressing hepatic lipogenesis through reducing levels of SREBP-1c, upregulating fatty oxidation in the liver and skeletal muscle through PPAR activation, and enhancing flux of glucose to glycogen through downregulation of HNF-4α. The net result of these processes is the repartitioning of metabolic fuel from triacylglycerol storage toward oxidation, thereby reducing the substrate available for VLDL synthesis. By simultaneously downregulating genes encoding the proteins that stimulate lipid synthesis and upregulating genes encoding the proteins that stimulate fatty acid oxidation, n–3 fatty acids are more potent hypotriglyceridemic agents than are n–6 fatty acids on a carbon-for-carbon basis. Furthermore, as stated above peroxidation of n–3 fatty acids reduces VLDL secretion through stimulating apolipoprotein B degradation. The n–3 fatty acids may also act by enhancing postprandial chylomicron clearance through reduced VLDL secretion and by directly stimulating lipoprotein lipase activity. It is proposed that the use of fish oil is a valuable clinical tool for the treatment of hypertriglyceridemia (81).

Studies on the effects of fish oil on hyperlipidemic apolipoprotein (APO) E*3-Leiden mice, which have impaired chylomicron and VLDL remnant metabolism, also indicate that fish oil-fed mice show a significant dose-dependent decrease in serum cholesterol (up to –43%) and triacylglycerol levels (up to –60%), mainly due to a reduction of VLDL (–80%). VLDL-apoB kinetic studies indicate that fish oil feeding produces a significant twofold increase in VLDL-apoB fractional catabolic rate (FCR), but hepatic VLDL-apoB synthesis is not affected. VLDL–triacylglycerol turnover studies show that fish oil significantly decreases the rate of synthesis of hepatic VLDL–triacylglycerol (–60%). A significant increase in VLDL–triacylglycerol FCR is observed (70%), which is not related to elevation in lipolytic activity. It is suggested that APOE*3-Leiden mice are highly responsive to dietary fish oil and observed strong reduction in serum VLDL is primarily due to an effect of fish oil to decrease hepatic

VLDL–triacylglycerol production rate and to increase VLDL-apoB fractional catabolic rate (van Vlijmen et al., 1998). Collective evidence indicates that the consumption of fish oil is cardioprotective at doses >3 g/day. The cardioprotective effects are due to suppression of fatal arrhythmias rather than stabilization of atherosclerotic plaques. At doses >3 g/day, EPA plus DHA can improve cardiovascular disease risk factors, including decreasing plasma triacylglycerols, blood pressure, platelet aggregation, and inflammation, while improving vascular reactivity. Mainly on the basis of the results of randomized control trials, the American Heart Association recommends for everyone to eat oily fish twice per week and that those with coronary heart disease eat 1 g/day of EPA plus DHA from oily fish or supplements (82).

Effect of Fish Oil on Liver

Fish oil exerts hypolipidemic, antiobesity, and antiinflammatory effects on hepatic lipid metabolism. A slight increase in the serum activity of liver enzymes during n–3 fatty acid supplementation has been reported by several investigators. Treatment of obesity-prone C57BL/6 J mice with 8% fish oilhigh-fat (30%) diet for 5 months results in the loss of body weight compared to mice fed a 30% triacylglycerol diet without fish oil. In addition to modulating mRNA levels in the liver, fish oil ingestion for 2 weeks upregulates the intestinal mRNA levels of lipid metabolism-related genes such as carnitine palmitoyl transferase 1a, cytochrome P450 4A10, and malic enzyme, compared with mice that are fed with the 30% triacylglycerol diet. Northern blot analysis demonstrate that the expression levels of most lipid metabolism-related genes in the small intestine of mice fed with 8% fish oil diet are comparable to those in the liver. Furthermore, fish oil ingestion also modulates lipid metabolism-related enzyme activity, fatty acid b-oxidation, o-oxidation, and malic enzyme activities in the small intestine of mice fed the 8% fish oil diet compared to mice fed the 30% triacylglycerol-enriched diet. These findings suggest that an upregulation of intestinal lipid metabolism is associated with the antiobesity effect of fish oil (83). Microarray based expression profiling of genes in rats fed with fish oil and ethanol diet indicates that a large number of genes show significant changes in female livers compared to males. The upregulated genes in female liver are associated with proteosome endopeptidase activity, lipid metabolism, alcohol metabolism, mitochondrial and oxidoreductase activity. The downregulated genes are associated with oxidoreductase activity, chaperone activity, and electron transport activity in the female liver as shown by biological theme analysis. To identify specific regulatory networks of genes operative in promoting liver injury, ingenuity

computational pathway analysis has been used. These studies identify a large cluster of genes associated with lipid metabolism, development, cellular growth and proliferation, apoptosis, carcinogenesis, and various signaling pathways (84).

Diet enriched in fish and marine oils also improves serum lipid profiles by suppressing liver X receptor a (LXR a) activity in the liver. Studies on the effects of trans geometric isomers of eicosapentaenoic acid (TEPA) on triacylglycerol synthesis induced by a synthetic LXR a agonist (T0901317) on HepG2 cells indicate that TEPA significantly reduces the amount of cellular triacylglycerol and the expression of mRNAs encoding fatty acid synthase, stearoyl-CoA desaturase-1, and glycerol-3-phosphate acyltransferase induced by T0901317 compared with EPA. However, there was no significant difference between the suppressive effect of TEPA or EPA on the expression of sterol-regulatory element binding protein-1c (SREBP-1c) induced by T0901317. It is shown that TEPA, but not EPA, downregulates the mRNA expression of peroxisome proliferator-activated receptor g coactivator 1ß (PGC-1 b), which is a coactivator of both LXR a and SREBP-1. It is proposed that the hypolipidemic effect of TEPA can be attributed to a decrease not only in SREBP-1 but also in PGC-1 b expression (85). Collectively, these studies indicate that dietary fish oil modulates the fatty acid composition of hepatic membranes, plasma lipoprotein cholesterol profile, biliary lipids, and the expression of proteins involved in reverse cholesterol transport. Fish oil reduces plasma HDL cholesterol and increases biliary cholesterol without concomitant modifications in the expression of key genes and proteins involved in reverse cholesterol transport. These findings suggest that functional changes in the activity of these proteins as a consequence of the incorporation of n–3 fatty acids into hepatic membranes and plasma lipoproteins may underlie the effect of fish oil feeding on plasma and hepatic cholesterol metabolism in the rat
(86).

Chronic hyperglycemia is an additional risk factor for cardiovascular disease. Diabetes patients have two- to four fold increased risk of dying from complication of cardiovascular disease. Considerable attempts using drugs (statins, fibric acid derivatives, and nicotinic acid) are made to control the dyslipidemia that severely deteriorates the diabetes in patients. Studies on the effect of fish oil on glycemic control of streptozotocin (STZ; 90 mg/kg bodyweight)-treated type 1 diabetic rats, fish oil-treated or untreated nondiabetic rats have indicated that 3 weeks after fish oil treatment, plasma total cholesterol is reduced in both the non-diabetic and the diabetic rats by 35% and 10%, respectively. In non-diabetic and

diabetic rats, levels of triacylglycerol are decreased by 69% and 20%, respectively, compared with control rats. Fish oil treatment decreases non-esterified fatty acids (NEFA) by 29% in diabetic rats, but the NEFA levels in non-diabetic rats are not affected. After fish oil treatment in non-diabetic and diabetic rats, platelet aggregation is decreased by 49% and 37%, respectively, and total antioxidant status is increased by 18% and 17%, respectively. Insulin levels are increased by 27% in the fish oil-fed non-diabetic rats, whereas insulin levels are markedly decreased in diabetic rats. Fish oil treatment does not alter glucose levels, but it reduces fructosamine levels by 29% only in fish oil-fed diabetic rats (87). Collective evidence suggests that fish oil may ameliorate the atherogenic lipid profile, platelet hyperaggregation, and the antioxidative defence of STZ-diabetic rats, and amelioration is independent of the effects of fish oil on glycemic control. Similar results are obtained on noninsulin-dependent diabetic patients on supplementation of n–3 fatty acidsenriched diet. Based on these observations, it is proposed that fish oil treatment decreases vascular complications in diabetes (88).

n–3 Fatty Acids and Bleeding Tendency

The bleeding tendencies of Greenland Eskimos, who consume n–3 fatty acidenriched diet (≥7–10 g/day), have been described by several investigators. Bleeding tendencies include prolonged cutaneous, nose, urinary tract, and obstetric bleedings. Furthermore, Greenland Eskimos show reduced platelet in vitro aggregability, compared with Danish control subjects. This reduction in platelet function has been explained by a shift in eicosanoid metabolism when ARA (20:4n–6) is replaced by EPA (20:5n–3) in platelet membranes. This shift results in the synthesis of thromboxanes and prostacyclins, resulting in more vasodilatory and antiaggregatory hemostatic profiles. There is no clinical evidence of an increased bleeding tendency when individuals consumed moderately enriched n–3 PUFA supplemention (2–5 g/day). Collective evidence suggests that daily ingestion of moderate amount of fish oil reduces chances of chronic heart disease, stroke, liver, and kidney diseases independent of the type of fat present in the daily diet (89).

Effect of n–3 Fatty Acids on Blood Pressure

DHA-enriched diet attenuates the development of high blood pressure in spontaneously hypertensive rats. Incorporation of dietary DHA produces changes in the fatty acid composition of the vasculature and organs (aorta, renal artery, plasma, liver, heart, kidney, and lung) involved in the

maintenance of blood pressure regulation. It is proposed that alterations in fatty acid profiles of vasculature and organs affect systemic hemodynamics. The DHA-enriched diet markedly increases the levels of DHA in the aorta, renal artery, plasma, liver, heart, kidney, and lung by 5-, 15-, 7-, 6-, 3.8-, 3.5-, and 8.8-fold, respectively. The levels of EPA are also elevated, while there is a concomitant reduction in the levels of ARA, adrenic (AA), and docosapentaenoic acids (DPA). In addition, higher proportion of dihomo-g-linolenic acid and a lower proportion of ARA indicate the impairment in D5-desaturase activity. Treatment of DHA-treated stroke-prone spontaneously hypertensive rats (SHRSP) also causes a significant decrease in the levels of total cholesterol, LDL, triglycerides, lipid peroxide, serum creatinine, and blood urea nitrogen as compared with those in non-treated SHRSP, suggesting that DHA-mediated antihypertensive action may be associated with the amelioration of both serum lipid alteration and renal dysfunction in non-treated SHRSP. In addition, DHA-mediated alterations in biophysical properties of membrane and generation of docosanoids may also promote lowering of blood pressure in normal and hypertensive rats (90).

Recommendations for Intake of n-3 Fatty Acids

Commercial preparations of fish oil capsules are available over the counter in drug stores and supermarkets. An excessive use of n–3 fatty acids may produce harmful effects not only due to the effect on enzymic activities but also through their effect on membrane permeability. Recommendations for n–3 fatty acid intake have been made by several international scientific authorities. Thus, Health and Welfare Canada proposes the use of 1.0-1.8 g n–3 fatty acids/day. The International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends adequate intake of a minimum 0.22 g/day for n–3 fatty acids. British Nutrition Foundation (BNF) suggests a desirable dietary intake of 1.1 g and 1.4 g of n–3 fatty acids for females and males daily. FDA in the United States recommends the use of up to 3 g n–3 fatty acids/day (21).

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