Pleiotropic, Cardioprotective Effects of Omega-3 Polyunsaturated Fatty Acids

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Abstract: The cardioprotective effects of food rich in omega-3 (ω-3) polyunsaturated fatty acids (PUFA) on cardiovascular risk has been of interest from the moment when a low rate of coronary heart disease was documented in the Eskimo population. The aim of the present review is to discuss recent studies documenting multidirectional action of ω-3 PUFA due to its pleiotropic properties. Experimental studies in cellular and animal models have extensively documented the favorable effects of ω-3 PUFA (eicosapentaenoic acid and docosahexaenoic acid) on: inflammatory processes, endothelial dysfunction, platelet aggregation and arrhythmogenesis. It was reported that antiarrhythmic effects of ω-3 PUFA resulted from stabilization of cardiomyocyte membrane and inhibition of ion channels. Moreover, PUFA possess several pleiotropic properties i.e. anti-inflammatory, anti-atherogenic and antithrombotic. Anti-atherogenic effects (plaque stabilization) of ω-3 PUFA have recently been demonstrated. It was documented (OCEAN study) that eicosapentaenoic acid from a source of highly purified ethyl esters is incorporated into plaques in a relatively short period of time and these higher concentrations of ω-3 PUFA may stabilize vulnerable atherosclerotic plaques. The anti-inflammatory effect of ω-3 PUFA is associated with reduction of levels of TNF-α and interleukin-6. Eicosapentaenoic acid and docosahexaenoic acid inhibit arachidonic acid metabolism to inflammatory eicosanoids.

Key Words: Omega-3 polyunsaturated fatty acids, cardioprotection, pleiotropic effect.

1. INTRODUCTION

During the last 30 years it has been recognized that inhabitants living in some seaside areas (especially in islands) experienced cardioprotective benefit of omega-3 (ω-3) polyunsaturated fatty acid (PUFA) originating from fish-rich diet. For example, islanders from Greenland (Eskimoes) are characterized by a particularly low frequency of cardiac death [1]. The low rate of death related to coronary artery disease, observed among the Eskimoes is intriguing, especially taken together with the fact of extremely high ω-3 PUFAs content in their diet, which was estimated to be as high as 400 mg/day [2]. Similarly, low death rates were found in the Japanese, particularly in Okinawa island, where fish consumption is twice as high as in mainland Japan [3].

Comparing Eskimoes and Danes (living in the seaside territory, but not on the island), the former had lower levels of total cholesterol, low-density lipoprotein, triglycerides and higher high-density lipoprotein values [4]. These differences in the lipidogram can be explained by the fact that serum, obtained from Eskimoes, contained mainly omega-3 PUFAs, whereas the Danes had higher levels of ω-6 PUFAs [5], which probably is the result of dietary preferences. Interestingly, in Eskimoes who migrated and subsequently lived in Denmark, domination of ω-6 PUFAs was similar as in native Danes. Thus, diet composition appeared to be a stronger factor than the genetic background.

In fish-rich diet, two acids belonging to the ω-3 PUFAs series i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) play the cardioprotective role. These encouraging epidemiological observations prompted experimental studies. A group of Australian investigators reported that EPA and DHA prevented fatal cardiac arrhythmias in monkeys or rats with atherosclerosis developed from consuming a high saturated fat diet [6,7]. Therefore, ω-3 PUFA was considered as a therapeutic alternative for arrhythmia management in humans, potentially safer than drugs. Moreover, further studies showed that EPA and DHA possess several pleiotropic properties, i.e., anti-inflammatory, anti-atherogenic, antithrombotic and antioxidant [8-14].

The current paper reviews the mechanisms by which ω-3 PUFAs develop pleiotropic effect on cardiovascular system. Therefore, we have performed MEDLINE search (1966-April 2009) with terms: "omega-3 fatty acids" and "n-3 fatty acids".

2. STRUCTURAL CLASSIFICATION AND FUNCTION OF FATTY ACIDS

Fatty acids are classified by the length of the carbon chain (long chain, n-20 to 22; intermediate chain, n-18) and the number of double bonds (saturated, monounsaturated, polyunsaturated) [11].

Polyunsaturated fatty acids belong to 2 main classes: ω-6 PUFA and ω-3 PUFA (Fig. **1**). Ω-6 PUFAs are found mainly in vegetable oil whereas ω-3 PUFAs in fish, fish-eating animals or flaxseeds [9]. For PUFA, location of the first double bond relative to the $-CH_3$ or ω (n-) end is given. From the therapeutic point of view, it is fundamental observation that ω-3 PUFAs have got chemical structure similar to current anti-arrhythmic drugs. These agents consist of a long acyl hydrocarbon tail, more than 2 unsaturated carbon–carbon double bonds, and a free carboxyl group at one end [14].

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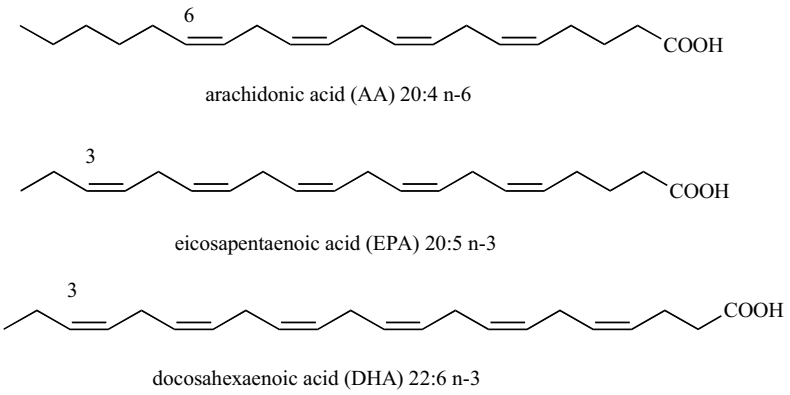

Fig. (1). Chemical structure of PUFAs.

As regards sources of PUFAs, long and intermediate-chain fatty acids must be ingested as part of the diet, because they cannot be synthesized by humans and therefore they are referred to be essential. The two most biologically active ω-3 PUFAs, i.e., EPA and DHA originate from an essential precursor molecule, α-linolenic acid [13]. This is a multistep synthesis. Firstly, α-linolenic acid is transported to the cells *via* specific fatty acid carrier and is converted by acyl-CoA synthases into thioesters. Then, these substances are used as substrates in cascade of reactions (β-oxidation, elongation, and desaturation) to synthesize lipids. At the final stage of this cascade, slow desaturation-elongation enzymatic process leads to generation of EPA and DHA.

Omega-6 PUFA is metabolized to form arachidonic acid, which maintains hemostatic balance in the system – however, only when released in small concentration. If ω-6 PUFAs is produced in large quantity, it can actively promote inflammatory process, trigger thrombosis and contribute in formation of atherosclerotic plaque [15].

Functionally, fatty acids form a powerful source of energy. They are the major energy source for the heart. Moreover, fatty acids are converted into bioactive eicosanoids (e.g., leukotrienes, prostaglandins, and thromboxanes). At the cell level, cardioprotective effects of ω-3 PUFAs are mediated by changes in membrane phospholipids structure, interference with eicosanoid intracellular signaling, and regulation of gene expression.

3. COMPETITION AND OPPOSING EFFECTS OF OMEGA-6 AND OMEGA-3 PUFA

The anti-inflammatory effects of ω-3 PUFAs are thought to be partially mediated by reduced synthesis of proinflammatory molecules from ω-6 PUFAs [13]. This is a competitive interrelation between both types of PUFAs, where ω-3 positively counterbalances negative action of ω-6 PUFAs. Unfortunately, ω-6 fatty acids are the predominant PUFAs in diet. Foods commonly eaten in present times contain ω-6 to ω-3 ratio in unfavorably high level 10:1 to 20:1, although a more optimal ratio may be closer to 1:1 [11,13,16]. The 1:1 ratio is difficult to achieve, since preferences of diet in current civilization, but even higher 4:1 ratio is still desirable and beneficial, because in the secondary prevention of cardiovascular disease, this reduced ratio (comparing to 20:1 ratio) was associated with a 70% decrease in total mortality [16].

The predominantly bioactive among ω-6 PUFAs is arachidonic acid (AA). Products of arachidonic acid metabolism include: prostaglandins, prostacycline, thromboxanes, leukotrienes, lipoxins, and epoxygenase products. The ω-3 fatty acid EPA is a competitive substrate for the enzymes of AA acid cascade. This alternatively substituted EPA in cascade reaction results in different end-products in "healthy pathway", many of which functionally oppose the products, resulting from AA metabolism [17]. In cellular structure, membrane ω-6 PUFA can be partially replaced with ω-3. When "friendly" EPA is available, "beneficial" thromboxane B_3 with its few physiologic effects is produced, rather than thromboxane B_2 - a potent vasoconstrictor and platelet activator. Available EPA further counteracts the adverse effects of thromboxane B_2 through the synthesis of prostaglandins that, along with those manufactured from AA, inhibit platelet aggregation and promote vasodilatation [18]. Omega-3 PUFA may also oppose ω-6 fatty acid metabolites by promoting the production of predominantly inactive leukotriene B_5 and competitively inhibiting the production of highly inflammatory leukotriene B_4 from AA. Altering the balance of downstream products from ω-6 and ω-3 fatty acids could also influence the arrhythmia threshold, since almost all the prostaglandins produced from AA are proarrhythmic, whereas those produced from EPA are not [19].

Biochemical processes regulate delicate balance between ω-3 and ω-6 PUFAs, since these PUFAs are powerful regulators of cellular functions with inflammatory, atherogenic and prothrombotic effects.

4. ANTI-INFLAMMATORY EFFECT OF PUFA

PUFAs modulate the generation of eicosanoids from AA. EPA acts as a substrate for the generation of "alternative" eicosanoids. Accordingly, substitution by EPA results in the production of less inflammatory and chemotactic eicosanoids than those, derived from AA [20,21].

DHA can reduce inflammatory signaling associated with increased cellular Ca^{2+} and activation of nuclear factor – kappa B (NF-□B) in human neutrophils [22]. Activation of NF-□B in neutrophils by intracellular Ca^{2+}-dependent mecha-

nisms also provides a potential rationale for linking Ca^{2+} inhibitory and anti-inflammatory properties of ω-3 PUFAs [22,23].

Recently, in a series of experimental models as a short-term studies, novel group of mediators has been identified, i.e., E-series resolvins, formed from EPA by cyclooxygenase-2 (COX-2). They appear to exert anti-inflammatory actions [24-30]. In addition, DHA-derived mediators, termed D-series resolvins, docosatrienes and neuroprotectins, also produced by COX-2, have been identified. They also appear to be anti-inflammatory [27-30]. Resolvin E_1 is an oxidized derivative of EPA that reduces inflammation by suppressing the activation of NF-κB and consequently the synthesis of inflammatory cytokines and chemokines.

In humans study, the beneficial effect of combined dietary intervention with ω-3 PUFA and plant sterols has been reported to reduce systemic inflammation in hyperlipidemic patients [31]. The authors suggest [31] that blunting inflammation provides a potential defense mechanism by which the combination of ω-3 PUFA and plant sterols are cardioprotective. This combined dual therapy in a 3-week, randomized, double-blind, placebo-controlled study reduced the level of several inflammatory markers. Accordingly, high sensitivity C-reactive protein was reduced by 39%, tumor necrosis factor-α (TNF- α) by 10%, interleukin-6 by 10.7%, leukotriene B_4 by 29.5% and adiponectin was beneficially increased by 29.5%.

As previously stated, food commonly eaten at present contains an ω-6 to ω-3 ratio in unfavorably high level 10:1 to 20:1, although a more optimal ratio may be closer to 1:1 [11,13,16]. To test inflammatory status in differently composed diets, the animal model of atherosclerosis (low-density lipoprotein receptor - knockout mice) was used. The authors [32] assessed the effect of different ratios of dietary ω-6 PUFA to EPA+DHA (ω-6:EPA+DHA) on atherogenesis and inflammatory response. The induction of atherogenesis was assessed in the following mice models: mice fed 32 weeks with high saturated fat diets without EPA and DHA or with omega-6:EPA+DHA at ratios of 20:1, 4:1 and 1:1. Mice fed with the lowest ω-6:EPA+DHA ratio diet had lower circulating concentrations of non-high-density lipoprotein cholesterol (25%) and interleukin-6 (44%), compared to mice fed with only the high saturated fat ω-6 diet. Elicited peritoneal macrophage total cholesterol was 25% lower in model of 1:1 diet, compared to only ω-6 high saturated fat fed mice. Monocyte chemotactive protein-1 mRNA levels and secretion were 37% and 38% lower, respectively, in elicited peritoneal macrophage isolated from animals on 1:1 diet compared, to ω-6 high saturated fat fed mice. mRNA levels of TNF-α were significantly lower in elicited peritoneal macrophage isolated from mice fed with 1:1 diet.

The anti-inflammatory effect of ω-3 PUFA has been documented recently not only in cardiovascular system but also in chronic inflammatory diseases, i.e., lupus erythematosus, asthma and Crohn disease.

5. ANTI-ATHEROSCLEROTIC AND VASOPROTECTIVE EFFECT

The initiating incident that triggers occlusion of a blood vessel and a thrombotic clinical event is a rupture of unstable atheromatous plaque. Omega-3 PUFA may actively contribute to cellular mechanism of atherosclerosis development. This effect was investigated both in experimental models and human studies.

The molecular mechanisms responsible for the favorable modulator effect of ω-3 PUFA on atherosclerotic process have been extensively studied in experimental models [33]. On the cellular level, ω-3 PUFA decrease production of several proatherosclerotic mediators: adhesion molecules [34], vascular smooth muscle cell growth factor [35], and chemoattractant molecules [36]. Consequently, ω-3 PUFA can blunt the destructive process of cell migration into the vessel wall intima that leads to atherosclerotic plaque formation. Furthermore, ω-3 PUFA might be able to protect endothelial layer, through attenuation of oxidative stress [10] - a powerful player in the development and progression of atherosclerotic vascular lesions [37]. This vasoprotective antioxidant effect was documented in cultured endothelial cells, pretreated with ω-3 PUFA and subsequently stimulated by specific cytokines or lipopolysaccharide [38]. Pretreatment with DHA beneficially blunted the endothelial response to the pro-inflammatory stimulation in terms of vascular cell adhesion molecule-1 (VCAM-1) cellular expression or release of macrophage colony stimulating factor (M-CSF). These effects were associated with decreased oxidative stress. Accordingly, PUFA supplementation studies providing 3.1 – 8.4 g EPA+DHA/day have reported 30–55% decreases in the production of reactive oxygen species (superoxide or hydrogen peroxide) by stimulated human neutrophils [39-41].

Other studies demonstrated that preincubation with DHA decreases in a dose-dependent manner the cytokine-stimulated *in vitro* expression of VCAM-1 by endothelial cells [42] and decreases the adhesion of monocytes to endothelial cells [43]. Similar, albeit weaker responses were observed also when cells were pretreated with EPA [43]. In animal model of atherosclerosis, EPA may potentially reduce and stabilize atherosclerotic lesions through its anti-inflammatory effects [44].

In humans, the randomized, placebo-controlled OCEAN trial plays a major role [45]. This study includes only surrogate, but not clinical endpoints. It was conducted to examine the effects of highly purified ω-3 PUFA ethyl esters on the composition and stability of atherosclerotic plaques among patients undergoing carotid endarterectomies. Important and practical reasons for selecting this type of patient included the opportunity to obtain large quantities of plaque. In this methodologically perfect study 121 adult patients, whose surgery was not scheduled during the next 7 days, were randomized to placebo or to 2 g/day of highly purified ω-3 PUFA ethyl esters. Median duration of study therapy was 21 days. The incorporation of ω-3 PUFA into excised plaque was assessed. Importantly, EPA was incorporated into plaques despite a relatively short period of treatment time. Uptake of EPA into plaques was substantial, more than doubled from baseline ($p<0.001$). In contrast, the increase in levels of DHA was relatively small (<20% *vs*. placebo) and not statistically significant. Detailed analyses revealed that plaque EPA content correlated negatively with indices of plaque inflammation and stability. Accordingly, the findings indicated that the higher concentration of EPA in the plaque

is associated with less inflammation, fewer macrophages and enhanced structural stability due to thicker fibrous caps. Accordingly, matrix metalloproteinase (MMP) enzyme expression within the plaque samples was assessed since MMP enzymes have been implicated in lysis of plaque caps, leading to plaque instability, erosion or rupture. The significant reduction of levels of mRNA for MMP-7, MMP-9 and MMP-12 was observed after treatment with ω-3 PUFA ethyl esters. There was also evidence for a substantial decrease in expression of intercellular cell adhesion molecule-1 (ICAM-1), which might contribute to a reduced plaque population of macrophages. Moreover, treatment with ω-3 PUFA ethyl esters was associated with reduced expression of mRNA for proinflammatory interleukin-6.

Endothelial dysfunction, defined as impaired endothelium-dependent vasodilatation, plays a pivotal role in early stages of atherogenesis. Several studies demonstrated improved endothelial function with a dose of EPA and/or DHA of least 3 g/day [46,47]. These effects of ω-3 fatty acids may be mediated through enhanced nitric oxide production [48]. An interesting issue is a potential link between biochemical and functional markers of endothelial dysfunction. In patients with peripheral arterial disease [49] ω-3 PUFAs 1 g b.i.d. for 3 months were added to the previous treatment. Endothelial function was assessed both biochemically (plasma soluble thrombomodulin) and functionally (brachial artery flow-mediated dilatation). Omega-3 PUFA beneficially reduced soluble thrombomodulin levels from the median value of 33.0 ng/mL to 17.0 ng/mL and improved brachial artery flow-mediated dilatation from 6.7% to 10.0%. The flow-mediated vasodilatation by 10% is a functional marker of restored (normalized) vasodilator response, typical for healthy subjects.

Influence on Lipemic Profile

Multipotential effect of ω-3 PUFA on lipid metabolism has been demonstrated [13]. EPA and DHA have been shown to increase intracellular degradation of apolipoprotein B-100 –containing lipoproteins [13]. This causes severe inhibition of secretion of very-low-density lipoprotein and thereby lowers plasma triglyceride levels. Combined EPA with DHA supplementation also appears to accelerate chylomicron triglyceride clearance by increasing lipoprotein lipase activity [50] and conversion of very-low-density to low-density lipoprotein [51], decreasing low-density lipoprotein synthesis, and reducing postprandial lipemia [52]. In clinical practice EPA and DHA are useful for treating severe hypertriglyceridemia and the beneficial effect is dose-dependent. In two different protocols of treatment with 4 g/day of ω-3 PUFA for 1 month versus 1 g/day for 6 months [53] triglycerides were decreased by 23% exclusively with the high-dose. In contrast, total and low-density lipoprotein cholesterol were not significantly affected by ω-3 PUFA in both studies.

The hypolipidemic, anti-atherogenic, anti-inflammatory effects of ω-3 PUFA may be enhanced by taurine [54].

Hypotensive and Anti-Diabetic Effect

In rat model with hypertension mediated by angiotensin II [55], the data suggest that ω-3 PUFA may reduce hypertension *via* rennin-angiotensin system (namely by interaction with angiotensin II). Metabolites of EPA, such as vasodilator eicosanoids, may counteract vasoconstriction induced by angiotensin II infusion. Additionally, ω-3 PUFA ameliorate ANG II-enhanced oxidative stress at the endothelial level and block superoxide anion production.

The hypotensive response to PUFA treatment is dose-dependent. In hypertensive patients with hyperlipidemia the two protocols of PUFA treatment were compared, i.e., long-term low doses (1 g/day for 6 months) versus short-term high dose (4 g/day for 1 month) [53]. In the short-term administration of enhanced dose, blood pressure was significantly reduced (-10 mm Hg systolic; -7 mm Hg diastolic), whereas no significant changes were observed in the long-term. To assess effects of a broad spectrum of ω-3 PUFA doses, a meta-analysis of 36 randomized trials of fish-oil supplementation was performed [56]. There was a small, but significant reduction in blood pressure of 2 mm Hg systolic and 1.5 mm Hg diastolic with a median dose of 3.7 g/day of fish oil [56].

The most recent observations in experimental diabetes indicate a beneficial effect of high dietary intake of ω-3 PUFA on reducing both hypertension and damage, common for diabetic renal disease [57]. Renoprotective effect involves attenuation of diabetic-related increase in collagen type I and type IV, interleukin-6, monocyte chemotactive protein -1, transforming growth factor-β.

In human study [58] with type 2 diabetes mellitus, the serum fatty acids composition was independently related to endothelial function evaluated by serum endothelin-1. Saturated fatty acids were associated with endothelial dysfunction (high levels of endotelin-1), whereas ω-3 PUFAs had a protective role in endothelial function.

Influence on Hemostasis

High doses of marine ω-3 fatty acids increase bleeding time [59] (Table **1**). The effect of PUFA on fibrinogen level varied widely in 24 trials [13]. Approximately half of these studies showed a net increase in fibrinogen level with ω-3 fatty acid consumption. The remaining studies showed either a net decrease or no effect. Similarly, inconsistent effect of ω-3 PUFA was reported in studies assessing coagulation factors. No significant changes after ω-3 PUFA treatment in factor VII, factor VIII and von Willebrand factor were reported. Additionally, no effect on plasminogen activator inhibitor–1 [59] and on tissue-type plasminogen activator antigen [60] was observed.

In the most recent study using APOE2 knock-in mice [61], the hypocoagulant effect of ω-3 PUFA was not caused by reduced hepatic synthesis of coagulation factors, but rather resulted from retention of uncarboxylated coagulation factors. The lipid-lowering effect of ω-3 PUFA links to altered expression of genes that regulate transcription and fatty acid metabolism.

In humans, ω-3 PUFA may play a supplementary role in statin therapy. It has been shown that ω-3 PUFA given in addition to simvastatin in patients with combined hyperlipemia, may reduce the tissue factor pathway inhibitor, a potent blocker of tissue factor- mediated activation of hemo-

Table 1. Ω-3 PUFA Effects on Hemostasis

Bleeding time	↑
Fibrinogen	+/-
Factor VII	+/-
Factor VIII	+/-
Von Willebrand factor	+/-
plasminogen activator inhibitor–1	+/-
tissue-type plasminogen activator antigen	+/-
Ω-3 PUFA+ simvastatin	
tissue factor pathway inhibitor	↓

static cascade. It plays a stabilizing role in atheromatic lesions, and reduces the prothrombotic activation, present in the postprandial phase [62].

6. ANTI-ARRHYTHMIC MECHANISM OF PUFA

The impressive decrease in risk of sudden cardiac death, associated with fish consumption or ω-3 PUFA treatment, suggests that these agents may have anti-arrhythmic properties underlying these cardioprotective effects. Anti-arrhythmic mechanisms on the cellular level may be divided into several groups of action [8,14,63-65]:

- counterbalancing interaction between ω-6 and ω-3 PUFA, direct membrane effects
- functional modulation of ion channels and exchangers (supplementation of ω-3 PUFA may have profound effects on trafficking of ion channels through subcellular compartments and in lipid rafts [70])

Restructuration of Cell Membrane

The particularly interesting concept is competitive balancing effect of ω-6/ω-3 PUFA ratio in arrhythmic status. Accordingly, incorporation of more ω-3 PUFA into cardiac membrane phospholipids favorably reduces this ratio, which may protectively shift the myocardium from proarrhythmic to anti-arrhythmic state [65]. Dietary supplementation causes a replacement of lipid membranes with enhancement of resident ω-3 PUFA components [66-68] and reduction in thromboxane B_2 [69]. Direct membrane effect is related to reordering of lipid membranes, with enhancement of resident ω-3 PUFA components. For example, in rats 10% fish oil can double the content of DHA in cardiomyocyte membrane [69]. In another study with rats, ω-3 PUFA re-ordered myocardial lipids pool and reduced arrhythmia susceptibility [68]

Sodium Channels

Evidence from animal models suggests that ion channel–mediated effects of PUFAs can exert anti-arrhythmic protection regulated *via* several mechanisms [70-73]. These effects may be mediated at least partially through a pathway connected with actions on the sodium channel, which underlie the excitation of cardiomyocyte in the ventricular myocardium. Functionally, the cardiac sodium current is responsible for the upstroke of the action potential and plays an important role in impulse conduction.

In more detail, the specific molecular mechanism of PUFA includes direct effects on voltage-gated sodium channels, causing hyperpolarization of the resting (diastolic) membrane potential and increased threshold voltage by 40% to 50% for the opening of the sodium channel [14,74,75]. PUFA-mediated changes in G-protein regulation of sodium channel function should be also considered [76]. Furthermore, the possibility that PUFA-induced changes in the sodium current could alter sodium homeostasis in the myocyte and thus indirectly change pH regulation, contractility or diastolic excitability, needs to be evaluated.

The data assessing ω-3 PUFA inhibitory potency are somewhat inconsistent and at times conflicting due to some factors, i.e., dose dependence of the ω-3 PUFAs effect. In the experimental model, using rat heart exposed to low concentrations of DHA, this fatty acid caused an increased plateau height, lengthening of the action potential duration, and a positive inotropic effect. In contrast, at higher concentrations in the rat myocardium (and at all levels that were tested in the guinea pig myocardium), the same agent reduced excitability and contractility.

From the practical viewpoint of clinical application it is essential to compare ω-3 PUFA action with anti(pro-)arrhythmic drugs class I. Importantly, ω-3 PUFA effects differ from infamous and even dangerous drugs (CAST study) because EPA did not up regulate cardiac sodium channels. On the contrary, it reduced the increase in sodium channel expression observed with mexiletine by 40 to 50% [77]. It was an important observation since the disappointing proarrhythmic toxicity of class I anti-arrhythmic drugs is associated with up regulation of sodium channel expression.

Potassium Repolarizing Currents

Both DHA and EPA can inhibit a number of various K^+ currents, and this effect may contribute to reports of cardiovascular protection [78,79]. According to the cell electrophysiology concept, the slow and rapid components of the delayed rectifier current are responsible for rapid repolarization of the action potential. The inward rectifier current contributes to the terminal phase of repolarization and to the maintenance of the resting membrane potential. A reduction of rapid inward K^+ current after acute administration of ω-3 PUFAs may explain why they prolong the action potential, as less repolarizing current is present during the repolarization phase of the action potential. On the other hand, increase of inward slow current by acutely administered ω-3 PUFAs leads to increased repolarizing current during the repolarization phase of the action potential. Whether the observed changes in repolarizing K^+ currents caused by ω-3 PUFAs lead to action potential prolongation or shortening will largely depend on the delicate balance between these and other depolarizing and repolarizing currents, species-differences regarding channel protein expression and the concentration of EPA and DHA [80]. The inhibitory action of ω-3 PUFA may involve, or even need, prior peroxidation and probably the redox status within the myocardium may modulate some of the electrophysiological effects of ω-3 PUFAs [81].

Omega-3 PUFAs are treatable option in two channelopathies-like diseases (dependent on K^+ current): epileptic seizures [82] and cardiac arrhythmia. Recently, it has been documented [83] that ω-3 PUFA affected voltage dependence of the shaker K^+ channel. The regulatory effects of ω-3 PUFA involve shifting the conductance versus voltage and the gating charge versus voltage curves in negative direction along the voltage axis. Uncharged methyl esters of the PUFAs did not affect the voltage dependence, whereas changes of pH and charge mutations on the channel surface affected the degree of the shifts. This finding indicates an electrostatic effect of ω-3 PUFA on the channel's voltage sensors. In contrast, monounsaturated and saturated fatty acids, as well as trans-PUFAs did not affect the voltage dependence. Summing up, only fatty acid tails with two or more *cis* double bonds are potent to place the negative carboxylate charge of the PUFA in a position to affect the channel's voltage dependence. It may be concluded that charged lipophilic compounds electrostatically regulate channel's voltage sensor.

Calcium Currents and Homeostasis

Intracellular calcium (Ca^{2+}) handling plays a regulating role and contributes to induction of triggered activity. The L-type Ca^{2+} current regulates the *plateau* phase and influences duration of the ventricular action potential. Acute administration of ω-3 PUFAs to ventricular myocytes reduces Ca^{2+} current [84,85] and thereby lowers the *plateau* level of the action potential. Incorporated ω-3 PUFAs into cellular membrane also reduce Ca^{2+} current and, more importantly, block "reopening" of the calcium channel at *plateau* potentials. This extra-inhibitive activity prevent dangerous arrhythmic events: early afterdepolarization and Torsade de Pointes (life-threatening subtype of ventricular tachycardia) [86]. Omega-3 PUFA inhibit the L-type Ca^{2+} channels [86], which in turn prevent triggered arrhythmic after-potential discharges caused by excessive cytosolic free Ca^{2+} concentration and also can prolong the refractory period, phase 4 of the cardiac cycle [75,84,87].

There is the other mechanism of ω-3 PUFA anti-arrhythmic activity depending on prevention of cellular Ca^{2+} overload. The sarcoplasmic reticulum is the source of most cellular Ca^{2+} that directly activates myofilaments. Short-term application of ω-3 PUFA causes an increase in sarcoplasmic reticulum calcium content [88] and a reduction in ryanodine receptor openings [89,90]. Thus, ω-3 PUFA inhibit Ca^{2+} release from sarcoplasmic reticulum mediated *via* ryanodine receptors. However, dietary ω-3 fatty acid supplementation may not increase sarcoplasmic reticulum Ca^{2+} content [91].

Sodium–Calcium Exchanger

The sodium-calcium exchanger (NCX) operates by extruding calcium from the cytosol and facilitates diastolic relaxation between cycles of cardiac excitation. In quantitative assessment, the NCX exchanges 3 Na^+ ions for 1 Ca^{2+} ion and therefore NCX is able to induce bidirectional currents (inward or outward) and modulates the shape and duration of the action potential. The inhibitory potency was supported by experiments in ventricular myocytes, where both outward and inward NCX were reduced by approximately 60% in the presence of incorporated ω 3-PUFAs [86].

In brief, cardiac electric activity is strongly modulated by ω-3 PUFA-related factors, including the metabolic state of the myocyte, the availability of oxygen and energy substrates (like plasma fatty acids and glucose) and the lipid composition of the cell membrane. The signaling pathways system in cell membrane plays a modulatory role *via* ion channels, exchangers, and pumps acting as complexes.

In clinical perspective, the mechanism of the anti-arrhythmic effect of ω-3 PUFA highlights the ability of these compounds to stabilize the electrophysiology of cardiomyocyte especially after a myocardial infarction.

7. LARGE, RANDOMIZED, PLACEBO-CONTROLLED CLINICAL STUDIES AND META-ANALYSES

It is probable that in patients with prior myocardial infarction, arrhythmias may have been generated on triggered activity, due to spontaneous Ca^{2+}-releases and prolonged action potentials [64]. Taking into consideration the mechanisms associated with post-infarction arrhythmogenic substrate, the increased intake of ω-3 PUFA supplements or fatty fish should be used. Accordingly, in the large secondary prevention clinical trial with fish oil, the GISSI–Prevenzione study [92], mortality due to sudden cardiac death was reduced by 53% and total mortality by 41% after myocardial infarction in patients treated with a relatively low, but clinically effective dose (1.0 g/day) of pharmaceutical-grade fish oil. From the statistical point of view it is essential that favorable divergence of survival curves for sudden cardiac death were observed very early, i.e., before 3 months after initiating fish oil therapy [93]. The reduced cardiovascular mortality after PUFA was also observed in patients with heart failure in GISSI-HF study [94].

Summing up clinical evidences, the most convincing support for anti-arrhythmic properties of fish oils has come from epidemiologic studies relating fish consumption to risk of sudden cardiac death, fatal myocardial infarction or mortality due to coronary artery disease. In line with the intuitive assumption, populations with low intrinsic levels of fish in their diets achieved most benefit. However, there are some controversies to resolve, because attempts to reproduce these findings in all clinical settings have not shown the same consistency in results (meta-analyses [95-100] presented in Table **2**). Disparities among these data reflect in part the relatively small numbers of previous studies, as well as substantial differences in the study protocols, including source, amount and method of administration of ω-3 PUFAs tested. The next important factors responsible for differences in results were duration of supplementation, varying degree of control of background diet (particularly intake of other fatty acids that may have proarrhythmic or anti-arrhythmic effects) and the end-points used to assess arrhythmias.

8. SIDE EFFECTS

Side effects are dose-dependent and may appear if doses are larger than 2 g/day (Table **3**). The side effects can be divided into medical (gastrointestinal, hemorrhagic and metabolic) or non-medical complication (fishy aftertaste). In the latest, large study GISSI-HF the rate of side effects was low and comparable with placebo (2,9%).

Table 2. Meta-Analyses on the Effect of ω-3 PUFA on Fatal and Non Fatal Cardiac Events

Study	Period of Data Collection/ Period of Follow-up	Number of Participants	Type of Omega-3 PUFA Source	Outcomes	Relative Risk (95% CI)
Whelton *et al.* [95]	1966-2003	228 864	Dietary	CAD mortality	0.83(0.76-0.90)*
He *at al.* [96]	Average 11.8 years	222 364	Dietary Fish intake 1-3/month 1/week 2-4/week 5≥week	CAD mortality	 0.89(0.79-1.01)* 0.85(0.76-0.96)* 0.77(0.66-0.89)* 0.62(0.46-0.82)*
Zhao *et al.* [97]	1996-2007	20 997		Angina pectoris Sudden death after MI cardiac death all causes of mortality	1.39(1.01-1.92) 0.43(0.20-0.91)* 0.71(0.50-1.00) 0.77(0.58-1.01)
Yezebe *et al.* [98]	1966-2003	14 727	Dietary or supplement	All causes of mortality Fatal MI Non fatal MI Non fatal stroke Angina pectoris	0.84(0.76-0.94)* 0.76(0.66-0.88)* 1.03(0.87-1.19) 1.36(0.87-2.29) 1.03(0.85-1.20)
Bucher *et al.* [99]	1966-1999	15 806	Dietary or supplement	Non fatal MI Fatal MI Sudden death Overall mortality	0.8(0.5-1.2) 0.7(0.6-0.8)* 0.7(0.6-0.9)* 0.8(0.7-0.9)*
Hooper *et al.* [100]	Before 2002	36 913	Dietary or supplement	Total mortality	0.87(0.73-1.03)

*- statistically significant, MI-myocardial infarction, CAD-coronary artery disease.

CONCLUSIONS AND PRACTICAL IMPLICATION

Omega-3 PUFAs have a pleiotropic impact on cardiac electric activity, structural elements of cell and functional regulation of ion channels and exchangers. EPA and DHA are competitive substrates for the enzymes and products of AA metabolism [101]. Very recently it has been reported [102] that some physiological effects of ω-3 PUFAs could partly result from a shift in the generation of active hydroxylated metabolites of AA through a catalysis mediated by cytochromes P450 from family 4.

The adequately high and properly composed ω-3 PUFA consumption of fish and fish oils appears in some large clinical trials to have beneficial effects on survival. Prevention from sudden death was observed particularly or at least in ischemic substrates (the origin of arrhythmogenic instability is important) and particularly or at least in populations with deficiency in consumption of fish. Essentially, evidence for a reduction in incidence of sudden death, in ischemic ventricular fibrillation, and in reperfusion ventricular fibrillation has been noted. Other anti-arrhythmic effects may also exist, such as in some circumstances of atrial fibrillation, representing supraventricular arrhythmia [103].

Clinical benefit from ω-3 PUFA has a dose ranging or dose-threshold effect. Appropriate formulation of EPA+ DHA also plays an important role. When fish oil supplements are taken, the variability in formulation, purity, and specific contaminants of the current over-the-counter preparations are likely to be a concern with respect to the effects. Accordingly, the effects may not be demonstrable in trials with too short duration, too low dose and/or suboptimal dosing compliance. The combination of PUFA used in GISSI-Prevenzione and GISSI-HF trials was as 1 g EPA plus DHA ethyl esters in a 46:38 ratio and administered as one capsule per day. Qualitative properties of Omacor differentiate it from other sources of ω-3 PUFAs, including higher content of DHA and the fact that both EPA and DHA are present as ethyl esters. They are pharmacokinetically distinct from the ω-3 triglycerides that are found in dietary sources and some other supplements, and these kinetic differences may be relevant to the clinical effectiveness of Omacor.

Table 3. Side Effects of Omega-3 PUFA

	Low dose <1 g/d	Moderate dose 1 g/d	High dose >3g/d
Gastrointestinal upset	+/-	+	+
Bleeding	+/-	+/-	+/-
Hyperglicemia*	+/-	+/-	+
Increase in LDL-cholesterol**	+/-	+	+

*usually only in predisposed patients, i.e., with impaired glucose tolerance or evident diabetes.

** usually only in patients with hypertriglyceridemia.

ABBREVIATIONS

PUFA = Polyunsaturated fatty acid

EPA = Eicosapentaenoic acid

DHA = Docosahexaenoic acid

AA = Arachidonic acid

MMP = Matrix metalloproteinase

NCX = Sodium-calcium exchanger

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