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Effects of omega-3 polyunsaturated fatty acids on cardiac myocyte protection

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1. ABSTRACT

Many epidemiologic, observational and randomized human clinical trials have demonstrated beyond doubt the protective cardiovascular effects of omega-3 polyunsaturated fatty acids (PUFAs). Cardiac myocytes protection by omega-3 PUFAs involves several mechanisms which might have a synergistic effect. This review provides a summary of the *in vitro* and *in vivo* effects of omega-3 PUFAs on cardiac myocytes health and reports the outcome of a number of clinical trials in patients consuming omega-3 PUFAs .

2. INTRODUCTION

The finding that death from ischemic heart diseases comprises only 3.5 % of all deaths in Greenland Eskimos who consume a diet rich in omega-3 polyunsaturated fatty acids (PUFAs) (1) has opened novel studies aiming at investigating whether the administration of omega-3 PUFAs in the diet could protect against death from heart diseases. The notion that omega-3 PUFAs exert cardiac myocytes protection has been supported by both *in vitro* and *in vivo* studies employing experimental models. In addition, many epidemiological, observational and

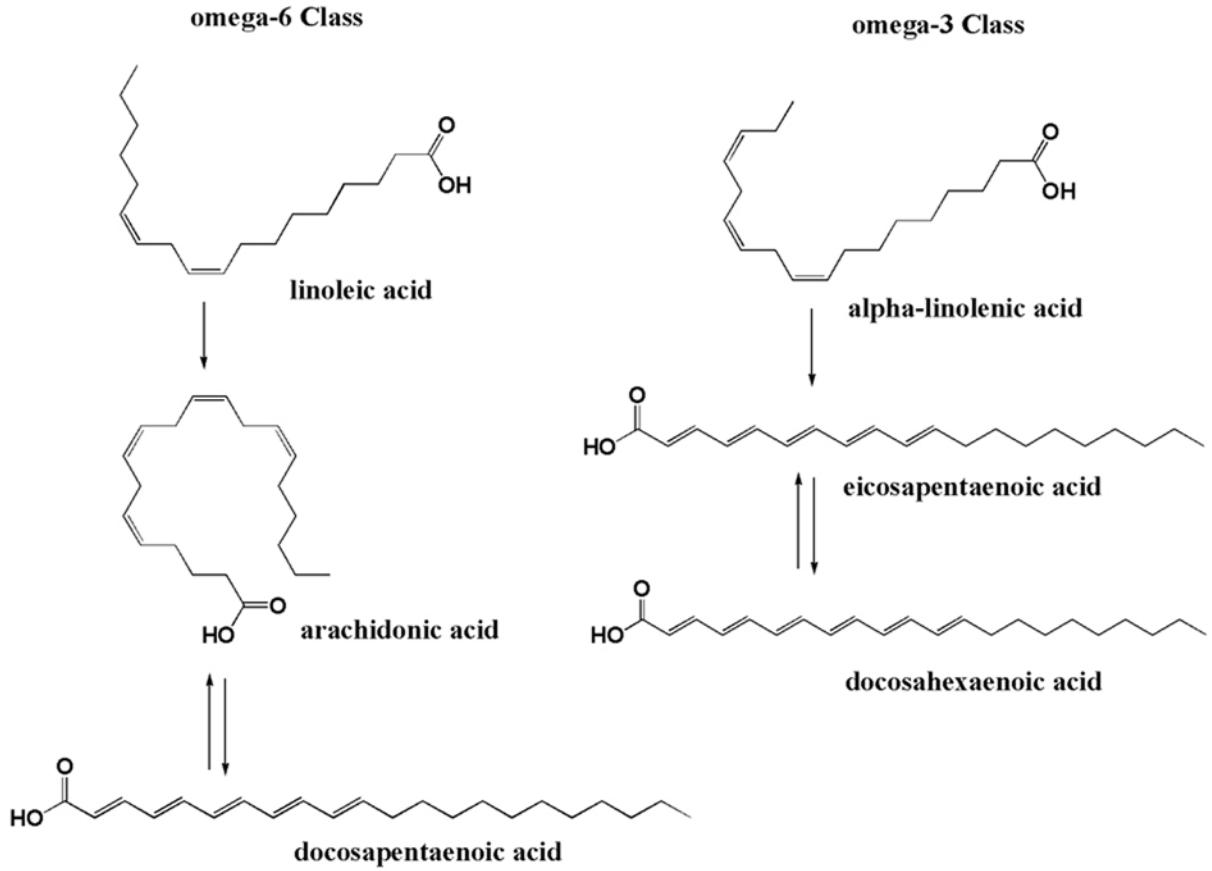

Figure 1. The metabolism of omega-3 and omega-6 PUFA.

randomized human clinical trials has undoubtedly demonstrated the cardiovascular protective effects of omega-3 PUFAs. Accordingly, the American Heart Association/American College of Cardiology recommends the use of omega-3 PUFAs for secondary prevention of cardiovascular disease for individuals with documented coronary artery disease (2). This review provides a summary of the *in vitro* and *in vivo* effect of omega-3 PUFAs on cardiac myocytes health and reports the outcome of a number of clinical trials in patients consuming omega-3 PUFAs .

3. OMEGA-3 PUFAs METABOLISM

n-3 (or omega-3) and n-6 (or omega-6) PUFAs are designated essential fatty acids because the lack of the Delta12- and Delta15-desaturases in humans prevents PUFAs' synthesis. For this reason PUFAs must be acquired from the diet (3, 4). Omega-3 and omega-6 PUFAs have both a long acyl chain of 18-22 carbon atoms but differ on the location of the first double bond encountered from the methyl end of the fatty acid (3, 4). Alpha-linolenic acid (ALA) and linolenic acid (LA) are the parent fatty acid of the omega-3 and the omega-6, respectively (Figure 1). LA, found in nuts, seeds and vegetable oils including corn, sunflower, soybean oil, can, once ingested be desaturated by Delta6 desaturase, elongated and desaturated by Delta5 desaturase to yield arachidonic acid (AA). ALA, found in seeds of flax, rape, perilla and chloroplasts of leafy green vegetables, can, upon ingestion be, metabolized by the same set of enzymes that convert omega-6 PUFAs, and then be converted into eicosapentaenoic acid (EPA), which can be further transformed into docosapentaenoic acid and finally into docosahexaenoic acid (DHA) (Figure 1). DHA placed on cell membrane phospholipids represents the main storage of omega-3 PUFAs in cardiomyocytes and neurons. ALA can be formed by desaturation of LA in chloroplasts of green leaves, plankton and algae. Vertebrates, however, do not have the desaturase which converts omega-6 to omega-3 PUFAs; omega-3 PUFAs enter the human food chain via the consumption of big fish, which in turn consume smaller fish which eat krill consuming plankton (3, 4). Omega-6 PUFAs are instead obtainable in our diet from plant seed oils especially from cooking and table oils, which hold more than 70 % of omega-6 fatty acid, i.e. LA (3, 4). In addition, omega-3 fatty acids can be obtained from some plaints oils containing ALA which include flax seed, soybean and canola oils (3, 4). AA can be either converted to docosapentaenoic acid or to eicosanoids by cyclooxygenase (COX) to yield 2-series prostaglandins and thromboxanes, 4-series leukotrienes, resolvins and lipoxins (3-5). EPA can be either converted to DHA or to eicosanoids by COX and lipoxygenase (LOX) to yield 3-series prostaglandins and thromboxanes as well as 5-series

leukotrienes, resolvins and lipoxins (5). Two forms of COX have been described: COX-1, which is constitutively expressed in the majority of cells and COX-2, which is expressed at low levels in most cells, although it is noticeably enhanced upon stimulation, mainly in immune cells, by a wide range of proinflammatory or mitogenic agents (6, 7). Eicosanoids can act to both promote and inhibit inflammation (7). Immune cells contain a high fraction of the omega-6 PUFAs, namely AA and a low fraction of omega-3 PUFAs, particularly EPA (8). The eicosanoids synthesized from AA are generally thought to exert a pro-inflammatory effect, while those derived from EPA are believed to have less inflammatory properties or even anti-inflammatory effects as compared to AA-derived eicosanoids (5, 6, 8). Since LA and ALA compete for the same enzymes, the consequence is that the surplus of one substrate determines a reduction of the other (5). Indeed, EPA and DHA compete both with AA for incorporation into cell membrane phospholipids and as substrates for COX, thus leading to an increase of 3-series prostaglandins (6). Accordingly, a high intake of omega-6 PUFAs with the diet would lead to a higher incorporation of AA in cell membrane as compared to EPA and DHA and a superior conversion of AA in eicosanoids with pro-inflammatory effects (3).

4. EFFECTS OF OMEGA-3 PUFAs ON CARDIAC MYOCYTES

In vitro and *in vivo* experimental models have been employed to demonstrate that omega-3 PUFAs affect the contractility of cardiac myocytes and have an anti-arrhythmic effect (3, 8-71). The key omega-3 PUFAs' mechanism of action in the prevention of cardiac arrhythmias relies on their effects on cardiac ion channels including: a) the voltage-gated Na^+ current (I_{Na}), which activation leads to a quick entrance of sodium ions and initiates action potentials; b) the voltage-gated L-type Ca^{2+} current ($I_{Ca,\ L}$), which is important for the Ca^{2+} release in the cytoplasm of cardiac myocytes, for electro-mechanical coupling and contraction of the heart and, c) K^+ channels (transient outward, I_{to}; inward rectifier I_K and I_{K1}; ultra-rapid delayed rectifier, I_{Kur}) (9). The opening of inwardly rectifying voltage-gated Na^+ channels initiate the action potential in heart. When electrical impulses from damaged cardiomyocytes alter the normal synchronicity of heart contraction an arrhythmia occurs. Hypoxia potentiates the persistent I_{Na} that may generate arrhythmias (10). Ventricular fibrillation represents the most common fatal arrhythmia. It was demonstrated that omega-3 PUFAs can decrease membrane electrical excitability by increasing the threshold for action potential, the resting-membrane potential, and the refractory-period duration in the cardiac myocytes (11). However, it was reported that prevention of arrhythmias by omega-3 PUFAs can also account for the interaction of various mechanisms including modification of eicosanoids and fatty acid membrane phospholipids composition, effect of non-sterified fatty acid (NEFA) on cardiac myocytes and finally effect of omega-3 PUFAs on the inositol lipid cycle, enzymes and receptors (12). The importance of these mechanisms in generating arrhythmias was supported by *in vitro* and *in vivo* studies. In addition, indirect effects of omega-3 PUFAs on cardioprotection include inhibition of inflammation, platelet aggregation and vasoconstriction as well as decrease of triacylglycerols (3)

4.1. *In vitro* effects of omega-3 PUFAs on cardiac myocytes: evidence of omega-3 PUFAs mechanisms of action

In earlier studies Hallaq *et al.* reported that the incorporation of EPA into cardiac myocytes cell membranes safeguards them from the fatal condition of contracture induced by of 0.1 mM ouabain by avoiding toxic levels of cytosolic Ca^{2+} (13). Later, it was demonstrated that perfusion of myocytes with EPA or DHA reduced, similarly to the antiarrhythmic drug lidocaine, the contraction rate of the cells without changing their amplitude of contraction (14).

The block of voltage-dependent sodium channels is considered a potent instrument for reducing cardiac myocyte excitability. Human cardiac Na^+ channels are composed of one alpha-subunit that produces a functional membrane channel and of a beta1-subunit, which regulates the voltage-dependent Na^+ channel (9). It was demonstrated that the acute addition of micromolar concentration of omega-3 PUFAs to adult rat myocytes rapidly blocked the Na^+ current and shifted the voltage dependence of inactivation to more hyperpolarized potentials. Among the others, DHA showed the best activity followed by EPA and ALA (15). Inhibition of I_{Na} by EPA was found to be dose, time and voltage dependent (16). EPA was shown to suppress $I_{Na,\ alpha}$ and to prolong the duration of recovery from inactivation (17), as well as to reduce $I_{Na,alpha\ beta}$, to accelerate channel transition from the resting state to the inactivated state and to extend the recovery time from inactivation (17). Similarly DHA and ALA inhibited both $I_{Na,alpha\ beta}$ and $I_{Na,\ alpha}$ (18). Xiao *et al.* found that asparagine at the 406 site in hH1alpha was important for PUFAs' inhibition of cardiac voltage-gated Na^+ currents (19). The same authors reported that a mutant of the alpha-subunit of human cardiac Na^+ channels determined a long-lasting persistent I_{Na} and that EPA inhibited I_{Na} in cells transfected with the inactivation-deficient mutant (10). Accordingly, Xiao *et al.* hypothesized a mechanism for the prevention of ischemia-induced arrhythmias by PUFAs. The authors assumed that during a myocardial infarction, myocytes depolarization can occur in the ischemic tissue. Then, central cardiac myocytes rapidly depolarize and die, while those in the periphery may be only incompletely depolarized and become hyperexcitable. Any other depolarizing stimulus can induce an action potential, which may lead to arrhythmia. However, omega-3 PUFAs induce elimination from activity of partially depolarized myocytes, thus aborting their potential arrhythmic property. Conversely, myocytes in the healthy myocardium with regular resting membrane potentials will not be prevented acting by PUFAs. These effects are thought to be mainly carried out by the effect of the omega-3 PUFAs on Na^+ and cardiac L-type Ca^{2+} channels (18). A model of interaction of PUFA with Na^+ channel proteins was proposed by Kang *et al.* The authors proposed a two-component model in which the lipophilic hydrocarbon chain of PUFAs allows their partitioning into the lipid layer and the interaction

with the hydrophobic transmembrane domains of the channel proteins, whereas the negative charge of the carboxyl group interacts with the positively charged amino acids residues of channel proteins near the surface of the bilayer (20).

Effects of omega-3 PUFAs on Ca^{+} channel have also been reported. It was found that extracellular application of EPA, DHA, ALA produced in rat ventricular myocytes a timely and reversible concentration-dependent suppression of $I_{Ca,L}$. EPA suppressed $I_{Ca,L}$ and Ca^{2+}-sparks, but it neither changed the temporal or spatial character of the Ca^{2+}-sparks, nor altered the ability of $I_{Ca,L}$ to trigger Ca^{2+}-sparks (21). In addition, in single, isolated ventricular myocytes from rat hearts, Negretti *et al.* demonstrated that the frequency of spontaneous waves of Ca^{2+} release and contraction was reduced in the presence of EPA (22).

Since the I_{to} and I_{Kur} play an important role in the repolarization of human atrium, an investigation was conducted on the effect of EPA and DHA on I_{to}, I_{Kur} and I_{Na}. The authors demonstrated that EPA and DHA inhibited I_{to}, I_{Kur} and I_{Na} in a concentration dependent manner (23). Earlier, it was shown the external blockade of the major cardiac delayed-rectifier K^{+} channel (Kv1.5) by DHA and AA (24). Besides, low concentrations of EPA and DHA inhibited the I_{to} without affecting other K^{+} currents and prolonged the action potential (25). Finally, it was reported that the I_{to} and the I_{K}, were both inhibited by the omega-3 PUFAs in adult ferret cardiomyocytes, while the I_{K1} were unaffected by the omega-3 PUFAs (26).

Omega-3 PUFAs were reported to additionally affect the behavior of other ion channels including the acetyl-choline-activated K^{+} channel ($I_{K,\ ACh}$), cardiac Na^{+}-Ca^{2+} exchanger (NCX1) and cAMP-activated cardiac chloride channel (9, 27). It was found that omega-3 PUFAs suppressed NCX1 (28). By using isolated myocytes from rabbits with heart failure and from patients with end-stage heart failure, it was demonstrated that acute administration of DHA and EPA inhibited the development of noradrenalin-induced triggered activity and diminished the number of delayed afterdepolarizations and calcium aftertransients by suppressing NCX1 (29). The meaning of omega-3 PUFAs in regulating the cardiac myocytes cation fluxes was also demonstrated in omega-3 PUFAs depleted rats, in which the phospholipids fatty acid membrane change composition was linked with alteration of cationic fluxes and the latter could be partially resolved by intravenous injection of omega-3- PUFAs rich fish oil emulsion (30).

Cardiac sarcolemmal plays a key role in regulating the exchange of ions in cardiac myocytes. However, the change of fatty acid composition of cardiomyocytes membrane can also affect the behavior of receptors as well as of transporters and enzymes inserted in the lipid layer. It was shown that cardiomyocytes membrane fluidity was increased following addition of DHA, EPA and ALA (15) and that the type of the long chain omega-3 PUFAs in the phospholipids played a role in the regulation of phosphatidylcholine-hydrolyzing phospholipase activity (31). Activation of the PKA pathway by EPA administration on rat ventricular myocytes was also proved. The authors hypothesized that this pathway activation has a positive lusitropic effect which could reduce diastolic dysfunction during ischemia, and promotes cell survival in ischemia by preserving ATP through a reduction of myofilament Ca^{2+} sensitivity (32). Picq *et al.* reported that omega-3 PUFAs could influence the level of cyclic nucleotide with increasing the cGMP basal level. Indeed, cardiac myocytes incubated with DHA or EPA supplemented medium decreased their cGMP-cyclic nucleotide phosphodiesterase specific activity (33).

By investigating whether the beneficial effects of omega-3 PUFAs could influence ischemia-reperfusion-induced alterations of myocardial alpha- and beta-adrenoceptor (alpha-AR, beta-AR) responsiveness, it was suggested that increasing omega-3 PUFAs in phospholipids reduced the increase in alpha- and beta-AR functional responses observed after hypoxia-reoxygenation (34). Grynberg *et al.* found that increasing the DHA content in membrane phospholipids of cardiomyocytes positively affected the beta-adrenergic transduction mechanism, through a cAMP efficiency increase (35).

The modification of cell membrane phospholipids and their effect on intracellular messengers (inositol(1,4,5)-trisphosphate (IP_3) and diacylglycerol) was investigated in isolated cardiac myocytes from adult pig hearts supplemented with EPA and DHA. It was established that after stimulation with epinephrine and phenylephrine, the levels of IP_3 in EPA and DHA supplemented myocytes were reduced in association with diminished levels of protein kinase C (36). Delerive *et al.* evaluated the influence of hypoxia duration and PUFAs composition on the capacity of cultured rat cardiomyocytes in producing alpha- and beta-adrenergic messengers (IPs and cAMP). The authors demonstrated a "beta-blocking-like" effect of omega-3 PUFAs (37).

Recently, evidence for direct TXA_2-induced cardiac arrhythmias was provided (38). Besides, it had been previously demonstrated that a selective thromboxane synthetase inhibitor in anaesthetized greyhounds increased survival following artery reperfusion (39). In light of these findings and given that EPA and DHA compete with AA for incorporation into cell membrane phospholipids and as substrates for COX, it appears that a reduced ratio of AA/EPA can reduce production of TXA_2 (12). Indeed, it was found that under normoxia, the eicosanoids production in cardiac myocytes depended on the cell phospholipids' AA content, while during posthypoxic reoxygenation the production of eicosanoids depended on the cell's omega-3 PUFA amount (40). In addition, Abeywardena *et al.* showed that the amount of the AA for TXA_2 biosynthesis depended on the NEFA pool and that omega-3 PUFAs replaced AA of this pool as well as inhibited the TXA_2 synthetase enzyme complex (41).

It was reported that excess of free fatty acid could be associated with the development of ventricular arrhythmias and death during acute myocardial infarction

(42). Although earlier Opie *et al.* reported the failure of high concentrations of circulating free fatty acids to induce arrhythmias in experimental myocardial infarction (43), Nair *et al.* demonstrated a specific modifications of phosphatidylinositol and NEFA fractions in cultured porcine cardiomyocytes supplemented with omega-3 PUFAs (44).

Omega-3 PUFAs supplementation was additionally demonstrated to have a direct control of gene expression in cardiomyocytes. In rat neonatal cardiac cells supplemented with omega-3 PUFAs it was shown the induction of several genes encoding proteins involved in lipid transport and metabolism and the down regulation of genes related to inflammation, cell growth, extracellular and cardiac matrix remodeling, calcium movements and ROS generation (45). In addition, the supplementation of primary cultures of rat cardiomyocytes with omega-3 PUFAs increased peroxisome proliferator receptor (PPAR) beta/delta to DNA and severely changed the acyl composition of both cytosolic and nuclear fractions (46). Furthermore, omega-3 PUFAs selectively accumulated in the nucleus and increased Acyl-CoA thioesterase activity, which catalyzes the reaction leading to NEFA from acyl-CoA (46).

Omega-3 PUFAs can also play an important role in cardioprotection. EPA supplementation was able to protect cardiomyocytes against palmitate-induced apoptosis (47). EPA and AA protected neonatal cardiac myocytes from ischemia/reperfusion-induced apoptosis through activation of ERK as well as induction of a dual-specific phosphatase, causing dephosphorylation of the pro-apoptotic kinase, p38 (48). Durot *et al.* reported that, in physiological conditions, the change of long chain PUFA composition in the cardiac muscle cells phospholipids could modify the initial action potential upstroke controlled by Na^+ channels. The presence of omega-3 PUFAs accelerates the electrical depression during substrate-free hypoxia and allowed a faster recovery upon reoxygenation (49). In addition, it was found that endothelin (ET-1)-induced cardiomyocytes remodeling could be prevented by pretreatment with EPA and that this effect appeared not to modulate the apoptosis signaling cascade (50). It was also found that EPA prevented ET-1-induced cardiomyocytes hypertrophy *in vitro* through the suppression of TGF-beta 1 and phosphorylated JNK (51).

Finally, omega-3 PUFAs were also shown to affect the property of several drugs. EPA was found to prevent and terminate in neonatal rat cardiac myocytes the contractures/tachyarrhythmia induced by isoproterenol which enhances cardiac Ca^{2+} and Na^+ channels (11). Chronic EPA treatment of neonatal rat cardiac myocytes was reported to reduce the mexiletine-induced increase in the cardiac Na^+ channel expression (52, 53). Pepe *et al.* showed that DHA inhibited the action of the Ca^{2+}-channel agonist BAY K8644 (dihydropyridine (DHP) agonist) and the Ca^{2+}-channel antagonist nitrendipine (NITR) (DHP antagonist) in rat isolated cardiac myocytes, but had no effect on beta-adrenergic receptor stimulation (54).

4.2. *In vivo* effects of omega-3 PUFAs in animals infused or fed omega-3 PUFAs

Early studies indicated that ventricular myocardial function in the rat was increased by the ingestion of animal saturated fat while polyunsaturated vegetable oil provided cardioprotection in some extent (55). Diets rich in omega-3 fatty acids from menhaden oil provided protection in a rat model of myocardial ischemia-reperfusion by producing variation in fatty acyl composition of membrane phospholipids in leukocytes, platelets and myocardial cells (56). In addition, cardiac sarcoplasmic reticulum from rats fed menhaden oil, had a lower content of saturated phospholipids, an increased DHA/AA ratio, and an increased ratio of omega-3 to omega-6 fatty acids. These changes were associated with a 30% decrease in calcium uptake and with a simultaneous decrease in membranes Ca-ATPase activity (57).

By investigating the ion channel characteristics of cardiac ventricular myocytes isolated from pigs fed for 8 weeks with a diet rich in fish oil containing omega-3 PUFAs, it was demonstrated increased omega-3 PUFAs content in the ventricular sarcolemmal which resulted in action potential reduction (58).

A fish oil diet in pig increased omega-3 PUFAs in the myocytes sarcolemmal, reduced incidence of E4031-induced EADs associated with a shorter action potential, reduced the action potential prolongation in response to E-4031 and reduced reactivation of L-type Ca^{2+} current (59).

Using a dog model of cardiac sudden death, it was proved that infused intravenously EPA, DHA or ALA just before the exercise-plus ischemia stress reduced the incidence of ventricular flutter-fibrillation (60). However, since omega-3 PUFAs could have altered ventricular myocyte calcium behavior, thus potentially affecting cardiac contractile function especially in myocardial infarction, the authors investigated the effect of dietary omega-3 PUFAs on ventricular function in dogs with healed anterior wall myocardial infarction (61). Dogs supplemented with DHA and EPA showed a omega-3 PUFAs increase in left ventricular tissue and red blood cells as well as reduction in heart rate and no alterations in ventricular mechanical function or L-type calcium current density and calcium transients (61). Conversely, Coronel *et al.* reported that a diet rich in fish oil resulted in proarrhythmia compared to a control diet during regional ischemia in pigs (62). On the other hand, McLennan *et al.* showed that feeding rats a diet supplemented with tuna fish oil significantly reduced the frequency and gravity of arrhythmias, preventing ventricular fibrillation during both occlusion and reperfusion of coronary (63). It was also described that dietary fish oil protected the rat heart against myocardial infarction and arrhythmias and improved postischemic recovery of heart activity when major coronary artery was occluded (64). The same research group showed that omega-3 PUFAs dose-dependently increased oxygen use and inhibited arrhythmias after saturated fat feeding in rats (65).

Fiaccavento *et al.* investigated the effect of an ALA-enriched versus standard diet in delta-sarcoglycan-null cardiomyopathic hamsters. Their findings demonstrated a great accumulation of ALA and EPA and increased EPA/AA ratio in cardiomyopathic hamster hearts, which correlated with the preservation of myocardial structure and activity. Indeed, ALA administration preserved cardiac myocytes plasmalemma and mitochondrial membrane integrity, thus maintaining proper cell/extracellular matrix contacts and signaling, as well as a normal gene expression profile (myosin heavy chain isoforms, atrial natriuretic peptide, transforming growth factor-beta1) and a limited extension of fibrotic areas. In addition, hemodynamic indexes were safeguarded, and more than 60% of ALA-fed animals were still alive while all those fed with standard diet died (66). In addition, Masuelli *et al.* demonstrated that the expression of N-cadherin, alpha- and beta-catenin was significantly reduced in cardiac myocyte intercalated disks of ALA-enriched versus standard diet in delta-sarcoglycan-null cardiomyopathic hamsters and was lowered to levels similar to those found in healthy hamsters. The authors also found that the cardiac myocyte intercalated disk ultrastructure was re-established in delta-sarcoglycan-null cardiomyopathic hamsters fed with the ALA-enriched diet (67).

O'Shea KM *et al.* provided evidence that dietary omega-3 fatty acids altered cardiac mitochondrial phospholipid composition and delayed Ca^{2+}-induced permeability transition pore (MPTP) of left ventricular in normal and infarcted myocardium of rats. Notably, the authors reported that dietary EPA + DHA increased the content of DHA and decreased the content of AA in subsarcolemmal (SSM) and interfibrillar (IFM). In addition, the ratio for DHA to AA was significantly increased in EPA + DHA-fed rats in both SSM and IFM (68). The authors highlighted the clinical usefulness of the delay in MPTP opening by dietary omega-3 fatty acids which it could translate into less myocardial injury in response to acute and chronic cardiac stress (e.g., ischemia/reperfusion or hypertension) (68). By analyzing the effects of dietary supplementation with EPA on ventricular arrhythmias during myocardial infarction in a canine model, it was showed that EPA supplementation increases the (Ca^{2+}-Mg^{2+})-ATPase activity within myocardial membranes which is involved in myocardial cells' Ca^{2+} metabolism by increasing the ratio of EPA to AA within cellular membranes (69). Suppression of IP_3 release by cardiac myocytes isolated from fish oil-fed pigs was also reported (70). Employing adult male Wistar rats, it was demonstrated that the combination of omega-3 PUFAs in the diet and chronic intermittent hypoxia (CIH) had a stronger antiarrhythmic effect during reperfusion than did the omega-3 PUFA in the di*et al*one. In addition, it was reported that lipid diets modified the extent of necrosis induced by CIH by a mechanism that required activation of the PKC delta-dependent pathway (71).

5. CLINICAL EVIDENCE IN HUMANS FOR OMEGA-3 PUFAs CARDIOPROTECTION

There is growing clinical evidence to support the cardioprotective effects of omega-3 PUFAs (72-75). The results of a number of these clinical trials are reported below. Earlier Burr *et al.* undertook a randomized controlled trial (Diet and Reinfarction trial-DART) to investigate the effect of dietary factors in the secondary prevention of myocardial infarction. 2003 men who had recovered from myocardial infarction were enrolled. The authors demonstrated that fatty fish and fish oil reduced mortality in men after myocardial infarction, by about 29% during the first 2 years (76).

The GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'infarto del miocardio) investigators performed a study in which 11,323 patients with recent ($\leq$3 months) myocardial infarction were enrolled in a multicenter, open-label, parallel, clinical trials with a follow duration of 3.5 year to test the efficacy of single or combined omega-3 PUFAs (1g/d) and vitamin E (300 mg/d). The authors documented that patients assigned to omega-3 PUFAs had a significant reduction of mortality very early in the course of the treatment mainly due to the decrease of sudden death (77). The GISSI investigators also performed a randomized, double-blind, placebo controlled trial in 326 cardiology and 31 centers in Italy. They enrolled patients with chronic heart failure of New York Heart Association class II-IV who were allocated in the omega-3 PUFAs 1g daily (n=3494) or placebo (n=3481) group. Patients were followed up for a median of 3-9 years. They demonstrated that omega-3 PUFAs were effective in reducing fatal events and hospital admission for cardiovascular causes and suggested that omega-3 PUFAs had an effect on the mechanisms leading to progression of heart failure (78). Harris *et al.* evaluated, by including twenty-five studies, the published associations between risk for coronary heart disease events and tissue omega-3 and omega-6 fatty acid composition. They observed that omega-3 PUFAs, especially DHA, were significantly reduced in patients occurring coronary heart disease (79). In the JELIS (Japan EPA lipid Intervention Study) trial 18,645 patients were randomly assigned to be given either 1800 mg of EPA daily with statin or statin only with a 5-year follow-up. In patients with no history or a history of coronary artery disease, the EPA treatment reduced by 18% and 19%, respectively, the major coronary events (80). Nodari *et al.* compared with placebo the effect of omega-3 PUFAs administration in 44 patients with idiopathic dilated cardiomyopathy and with frequent or repetitive ventricular arrhythmias at Holter monitoring. They demonstrated that omega-3 PUFAs administration had favorable effects on parameters related to the risk of malignant arrhythmias and sudden cardiac death. In addition, omega-3 PUFAs administration was also associated with a decrease in non-sustained ventricular tachycardia episode (NSVT) and in the heart rate of NSVT (81).

Leaf *et al.* in a prospective, randomized, placebo-controlled trial analyzed whether daily 2.5 g of EPA plus DHA would delay the time to first implanted cardioverter/defibrillators event for ventricular tachycardia or fibrillation or death during a 12-month period compared

with an olive oil control. The authors revealed that individuals with a high risk of fatal ventricular arrhythmias could reduce potentially fatal ventricular arrhythmias by habitual daily ingestion of fish oil fatty acids (82). Streppel *et al.* reported that long-term fish consumption, on average 22 g per day, decreased the risk of coronary heart disease death, especially below age 65. In addition, fatty-fish consumption decreased the risk of sudden coronary death but there was no clear dose–response relationship between EPA+DHA intake from fish and sudden coronary death (83). In the clinical trial performed by O'Keefe *et al.*, 18 white men with a history of myocardial infarction and ejection fractions <40% were randomized to placebo or omega-3 fatty acids for two 4-month periods. Omega-3 PUFAs decreased heart rate at rest and improved 1-minute heart rate recovery after exercise (84). Geelen *et al.* reported that supplementation with 1.5 g n-3 fatty acids/d from fish did not substantially control the number of premature ventricular complexes (PVCs) in patients with frequent PVCs, but decreased heart rate in a magnitude that predicted a lower risk of sudden death (85). Finally, Singer *et al.* performed a randomized, double-blind, placebo-controlled study in 65 patients with cardiac arrhythmias without coronary heart disease or heart failure. In the group (n = 33) supplemented with encapsulated fish oil over 6 months, a reduced incidence of atrial and ventricular premature complexes (86).

6. PERSPECTIVE

Omega-3 PUFAs have been demonstrated both in epidemiological and clinical trials to lower the frequency of cardiovascular diseases. Data from clinical trials sustain the recommendation made by the American Heart Association/American College of Cardiology to incorporate in the diet at least two portions of fish per week as well as vegetable oils and food rich with alpha-linolenic acid (72). The increase of omega-3 PUFAs intake in diet represents a food-based strategy to prevent cardiovascular diseases. However, since omega-3 and omega-6 PUFAs share the same metabolic pattern, it is conceivable that an excessive omega-6 PUFAs ingestion, typical of western diet, could limit or undo the beneficial effects of consuming omega-3 PUFAs. Evolutionary studies suggest that in the late paleolithic diet the dietary omega-3/omega-6 ratio was ~1, whereas in the current Western diet it is about 15:1. In the secondary prevention of cardiovascular diseases a omega-3/omega-6 ratio of 4:1 has been associated with a decrease of 70% in total mortality (87). Thus, much more important than any suggestion of overall increase of omega-3 PUFA consumption, would seem to be the control of the dietary omega-3/omega-6 ratio.

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