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Omega-3 Polyunsaturated Fatty Acid Supplementation: Mechanism and Current Evidence in Atrial Fibrillation

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Abstract

Atrial fibrillation (AF) is the most prevalent arrhythmia and is associated with considerable morbidity and mortality. Available pharmacologic antiarrhythmic therapies are often ineffective in preventing the recurrence of AF, possibly because these drugs target a single pathophysiological mechanism. Given their beneficial effects on ventricular arrhythmias, omega-3 polyunsaturated fatty acids (n-3 PUFAs) have recently been investigated as possible candidates in the treatment of supraventricular arrhythmias. In this review, we explore the current understanding of the antiarrhythmic effects attributed to n-3 PUFAs including direct modulation of ionic channels, improvement of membrane fluidity, anti-inflammatory and antifibrotic effects, and modulation of sympatho-vagal balance. We will then focus on the results of epidemiologic studies exploring the associations between nutritional intake of n3 PUFAs and the incidence of AF, and will review the findings of the clinical trials investigating the effects of n-3 PUFAs supplementation in the prophylaxis of AF and in the prevention of its recurrences.

Introduction

The prevalence of atrial Fibrillation (AF) is steadily increasing and represents a growing burden on the healthcare system. Over 6 million Europeans suffer from this arrhythmia, and the number of patients with AF in the USA is expected to reach between 5.6–15.9 million by 2050.[1,2] Moreover, AF occurs in approximately 25–30% of patients after isolated coronary artery bypass grafting (CABG), and in about 50% of patients after combined coronary artery and valvular surgery.[3] Post-operative AF is associated with a 2-fold increase in cardiovascular morbidity and mortality, largely due to stroke and circulatory failure.[4]

Numerous conditions such as advanced age, hypertension, diabetes, left atrial enlargement, ischemic heart disease, and congestive heart failure have been identified as risk factors for AF. On a pathophysiological standpoint, inflammation and oxidative stress have been recognized as pivotal mechanisms involved in the development, recurrence and persistence of AF, particularly in some specific forms such as post-operative AF .[5] Atrial fibrosis secondary to the inflammatory state represents the hallmark of arrhythmogenic structural remodeling, which plays an essential role in the initiation and in the perpetuation of AF.[6,7] Additionally, the persistence of AF itself may lead to changes in atrial myocyte metabolism and electrical properties, and eventually cause irreversible modifications of atrial structure and function.[7] The potential role in the treatment of AF of a va-

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riety of agents traditionally not considered antiarrhythmic but with anti-inflammatory and antioxidant properties has been explored in recent years.[8,9] In particular, omega-3 polyunsaturated fatty acids (n-3 PUFAs) have been in the front line, as they may target multiple pathogenetic pathways of AF. However, although the potential antiarrhythmic effects of n-3 PUFAs have been well demonstrated in experimental models of AF inducibility, the conflicting results obtained in clinical trials have been disappointing and have cast doubts and uncertainties regarding the efficacy of these drugs in the prophylaxis of AF and in the treatment of this arrhythmia.

Therefore, the aims of this paper are to review the experimental evidence underlying the mechanisms of the antiarrhythmic effects of n-3 PUFAs in AF, as well as to discuss the results of epidemiological studies exploring the association between n-3 PUFAs and AF, and the findings of clinical trials investigating the effects of n-3 PUFAs on the primary and secondary prevention of this arrhythmia. We will focus in particular on the potential explanations for the often conflicting results reported in the various trials.

Antiarrhythmic Effects of n-3 PUFAs: Mechanisms and Experimental Evidence

Studies conducted in cardiomyocytes in vitro, in isolated organs, and in animal models have helped to elucidate a number of mechanisms that may account for the antiarrhythmic effect of n-3 PUFAs in AF. In particular, n-3 PUFAs have been shown to: a) exert electrophysiologic effects; b) possess anti-inflammatory and antifibrotic actions; and c) affect the sympatho-vagal balance.

Electrophysiologic Effects of n-3 PUFAs

1.Modulation of Ionic Channels

n-3 PUFAs are essential component of the sarcolemma, where they modulate the interaction of the lipid bilayer with several membrane-associated structures. Additionally, n-3 PUFAs have been shown to affect ionic channels function, thereby increasing electrical stability. In particular, a decrease of L-type calcium (Ca++) currents and Na+/ Ca++ exchanger activity, and an increase of slow delayed rectifier potassium K+ currents appear to be the primary mechanism by which N-3PUFAs improve electrical stability.[10] Moreover, n-3 PUFAs inhibit the fast voltage-dependent Na+ current, increasing the depolarizing threshold potential for channel opening; as a consequence, a more intense depolarizing stimulus is required to elicit an action potential.[11]

2.Effects of n-3 PUFAs in Experimental Models of Atrial Fibrillation

Changes in the duration of the effective refractory period (ERP) appear to be an important early remodeling event favoring the development and perpetuation of AF.[12] In a canine model of rapid atrial stimulation, n-3 PUFAs administration significantly reduced the shortening of atrial ERP induced by rapid pacing, thus preventing acute electrophysiological remodeling.[13] Other experimental studies have demonstrated that n-3 PUFAs administration may influence the electrical membrane stability in isolated pulmonary vein (PV) preparation.[14,15] The modulation of membrane's ionic currents in the PV leads to shortening of the action potential, which may reduce spontaneous and triggered activity by decreasing the occurrence of early after depolarizations.[16] Experiments on isolated-perfused hearts from rabbits fed with DHA and EPA-rich diet have demonstrated that n-3 PUFAs may also improve membrane fluidity and reduce the stretch-induced drop in the refractory period.[17]

Anti-Inflammatory and Anti-Fibrotic Effects of n-3 PUFAs

Inflammation and abnormal oxidative stress seem to play a pathogenic role in the development, recurrence, and persistence of AF.[5] In two population-based studies, the presence of systemic inflammation, reflected by elevations in C-reactive protein (CRP), not only was associated with the presence of AF but also was able to identify patients at high risk for AF development .[18,19] This association between AF and CRP was independent of conventional risk factors such as hypertension, structural heart disease, previous stroke, or embolism. In a separate cohort, plasma levels of IL-6 were also found to be elevated in patients with chronic AF.[20] In a study conducted by Halonen et al. the administration of intravenous hydrocortisone reduced the incidence of AF after cardiac sur-

gery, suggesting that an increased inflammatory response could contribute to the development of postoperative AF.[21] In agreement with this evidence, the temporal course of AF occurring after cardiac surgery closely follows the activation of the complement system and the release of proinflammatory cytokines.[22] One of the mechanisms by which n-3 PUFAs modulate the inflammation is by decreasing the production of leukotriene B4 and of other inflammatory eicosanoids.[23] In addition, experimental evidence suggests that n-3 PUFAs may exert anti-inflammatory effects by activating the peroxisome proliferator-activated receptor (PPAR)-γ with consequent suppression of the synthesis of nuclear transcription factor (NF-kB) and NF-kB-regulated pro-inflammatory cytokines.[24] Moreover, in some animal models, n-3 PUFAs supplementation increases plasma levels of adiponectin,[25] an anti-inflammatory adipokine that activates the AMP activated-protein-kinase pathway and suppresses NF-kB signalling.[26]

Atrial fibrosis, the final result of the reactive responses to inflammation, stretch, oxidative stress, ageing, and myocyte apoptosis, represents a major feature of the structural and functional remodeling of the atrial myocardium and may interfere with conduction by impairing electrical coupling of the myocytes.[7] Electrical continuity between myocytes is maintained by gap junction proteins called Connexins (Cx). Abnormalities in the electrical coupling are considered an important arrhythmogenic factor, and an association between AF, increased atrial expression of Cx40 and Cx43, and remodeling of gap junctions has been documented.[27,28] The acute intravenous administration of n-3 PUFAs in a dog model of AF significantly reduced the vulnerability to induction of AF by the extra stimulus technique, and this effect was mostly related to reduced Cx40 expression.[29] Tissue fibrosis is the result of increased fibrillar collagen deposits, a process in which matrix metalloproteinases (MMP) play an important role.[7] In dogs exposed to simultaneous atrioventricular pacing to induce atrial remodeling, pretreatment with n-3 PUFAs was associated with lower AF vulnerability, which appeared to be related to a smaller increase in atrial MMP activity and collagen type I and III gene expression.[30] Additionally, in a dog model of pacing-induced atrial remodeling, n-3PUFAs supplementation started 7 days before pacing was associated with significant down-regulation of genes involved in fibrosis, hypertrophy, and inflammation, and with reduced susceptibility to AF.[31] Using the same experimental model of pacing-induced AF, these authors also found that n-3 PUFAs treatment, started 7 days after pacing, did not reduce the inducibility of AF and did not attenuate atrial remodeling or fibrosis.[32]

Effects of n-3 PUFAs on Sympatho-Vagal Balance.

A number of investigations have shown that n-3 PUFAs may favorably affect heart rate variability and baroreceptor reflex responses, suggesting a modulation of the balance between the sympathetic and vagal nerve control of the heart (sympatho-vagal balance).[33] We have previously reported that treatment with 1g daily of n-3 PUFAs in patients with idiopathic cardiomyopathy can exert positive modulatory effects on the sympathetic-vagal balance and significantly reduce circulating catecholamines and plasma levels of the inflammatory cytokine TNF-α, IL-6, and IL-1, with a reduction of heart rate and ventricular arrhythmias.[34]It is reasonable to speculate that these mechanisms might also play a favorable role in supraventricular arrhythmias. However, the ability of n-3 PUFAs to increase parasympathetic tone may theoretically exert pro-arrhythmic effects in younger individuals with normal heart, in whom a vagal component may play a role in promoting AF.[35]

Epidemiological and Interventional Studies: Conflicting Results and Possible Explanations

N-3PUFAs in Epidemiological Studies

In a seminal paper, Mozaffarian and colleagues investigated the associations between the consumption of tuna and other broiled or baked fish and the incidence of AF in the Cardiovascular Health Study (CHS), a population-based cohort study of 4,815 healthy subjects, aged 65 years and older. The authors reported that, at 12-year follow-up, higher fish intake was associated with a statistically significant 31% reduction of AF risk.[36] However, subsequent investigations did not consistently confirm this association (Table 1). These conflicting results may be explained, at least in part, by various factors such as difference in the age of the studied populations, lack of standard-

ized criteria for the diagnosis of AF, socio-economic and lifestyle differences, dietary changes during follow-up, and variable prevalence of underlying heart disease. Also, substantial geographic variation may exist in total fish intake and in intake of fatty fish, which may have affected the results in a given cohort. Moreover, while fish is considered a significant source of n-3 PUFAs in the diet, it is important to remember that there is a large variability in n-3 PUFAs content between different species of fish and that the mode of cooking may impact the nutritional value of fish, since no significant correlation exists between intake of fried fish and plasma n-3 PUFAs levels.[37] For instance, in a prospective cohort of 47,949 participants in the Danish Diet, Cancer and Health Study,[38] consumption of n-3 PUFAs from fish (herring, mackerel, sardine, trout, and salmon) not only was not associated with a reduction in the risk of AF, but an increased risk of AF was observed in the highest quintiles of fish intake when compared to the lowest quintile. The discrepancy with the results of the CHS may be due to the age difference between the study populations and the lower prevalence of cardiovascular risk factors in the Danish cohort. Furthermore, the latter study provides no information on how fish was cooked. Also in the Women's Health Initiative, a large cohort of healthy American women, no association between dietary intake of non-fried fish and incident AF was found during a 6-year follow up in the 44,720 participants not enrolled in the dietary modification intervention arm and without AF at baseline.[39] An analysis of 17,679 men with no history of cardiovascular disease enrolled in the US-based Physician's Health Study, showed that, at 15-year follow up, participants in the highest quintile of fish intake (≥5 meals per week) were more likely to develop AF compared with those in the lowest quintile. However, to date these findings have been published only in an abstract form.[40]

An important methodological note regarding all the studies described above, is their use of food frequency questionnaires to evaluate the dietary intake of n-3 PUFAs. Although these questionnaires provide a convenient means of estimating usual patterns of dietary intake, they are prone to several errors. For example, the frequency response options may not provide the most appropriate level of discrimination, the food list may be inadequate and questions regarding usual portion sizes may be ignored or estimated incorrectly.[41] To overcome some of the above limitations, in the Rotterdam Study[42] and in a recent analysis of the Framingham Health Study[43] investigators employed a more extensive, semi-quantitative food-frequency questionnaires in order to measure the intake of specific fatty acids. In the study by Brower and colleagues[42] the 5,184 participants were specifically asked to indicate the frequency, amount, and kind of fish eaten; intake of specific fatty acids was based on a food composition database derived from the TRANSFAIR study.[44] EPA plus DHA intake was categorized in tertiles of intake per day, and data analysis showed that an higher omega 3 intake was not associated with lower incidence of new onset AF at 6 year follow-up. Recently, Shen and colleagues using data from the Framingham Heart Study conducted longitudinal analysis to explore the association between dietary factors and incidence of new onset AF.[43] A total of 4526 selected participants were included in the final analyses and dietary fish intake was assessed by a validated 126-item semiquantitative FFQ.[45] Salmon, swordfish, bluefish, mackerel, and sardines were classified as dark fish, while canned tuna consumption was reported separately. Also in this study n-3 PUFAs intake from fish or fish-oil supplements not only was not associated with a lower incidence of AF, but the highest consumption of dark fish (> 4 servings/week) compared with the lowest consumption (< 1 serving/week), was associated with an increased AF risk. In both studies, however, despite using a more extensive and complete questionnaire, the main limitation of this fish intake assessment (i.e. the lack of direct measurement of n-3 PUFAs levels) was still present. Such dietary estimates also do not allow separate assessment of EPA and DHA intake. The impact of the use of biological measurements instead of a questionnaire in association studies is underscored by the data reported by Virtanen and colleagues, who measured serum n-3 PUFAs level as a marker of dietary fish intake in 2,174 male subjects enrolled in the Kuopio study.[46] This investigator found that higher concentration of DHA, but not of EPA, was associated with reduced risk of incident AF. More recently Wu and colleagues, in their analysis that included 3326 participant of the CHS[36] in which n-3 PUFAs plasma levels were measured, expanded these observations to an older population (mean age 74 years) that included a high proportion (60%) of women.[47] Results from

this large prospective study showed that, after adjustment for others risk factors and intervening events during follow-up (i.e. heart failure or myocardial infarction), higher total n-3 PUFAs and DHA plasma levels were associated with lower risk of incident AF. Additional analyses showed that this inverse association between higher N-3PUFAs plasma level and incident AF was minimally affected by additional adjustment for fish consumption, whereas the association between fish consumption and incident AF was attenuated after adjustment for EPA and DHA levels. These findings indicate that the direct measurement of circulating levels of n-3 PUFAs may provide a more objective method to evaluate the influence of n-3 PUFAs intake on the risk of AF than the indirect estimation based on the number of fish servings. In addition, direct measurement of circulating or membrane n-3 PUFAs levels appears to suggest possible different biological roles of EPA and DHA on

Table 1 Risk of Atrial Fibrillation and Fish Consumption in Population-Based Studies

Study (First author ref)	Study Population (Age population)	Country	Follow-up	Estimation of n-3 PUFAs Dietary Intake	Main Results (Risk of AF in the highest intake vs. the lowest intake group)
Cardiovascular Health Study (Mozaffarian 36)	4,815 subjects (≥ 65 years)	USA	12 years	Food Frequency Questionnaire	Lower AF risk with broiled fish RR 0.70 (95% CI; 0.53-0.91) p=0.008
Danish Study (Frost 38)	47,949 subjects (mean age 56 years)	Denmark	5.7 years	Food Frequency Questionnaire	Increased AF risk RR 1.34 (95% CI; 1.02-1.76) p = 0.001
Rotterdam study (Brouwer 42)	5,184 subjects	Netherlands	6.4 years	Semi-quantitative Food Frequency Questionnaire	No association RR 1.18 (95% CI; 0.88-1.57)
Physician's Health Study (Aizer 40)	17,679 male subjects	USA	15 years	Food Frequency Questionnaire	No association RR 1.46 (95% CI; 0.94-2.28)
Women's Health Initiative (Berry JD 39)	46,704 female subjects (50 to 79 years)	USA	3 years	Food frequency questionnaire	No association RR 1.01 (95% CI; 0.66-1.56)
Framingham Heart Study (Shen J 43)	9,640 subjects (4231 male; mean age 62 years)	USA	6 years	Semi-quantitative Food Frequency Questionnaire	No association RR 1.18 (95% CI: 0.85, 1.64) p = 0.57
Kuopio study (Virtanen JK 46)	2,174 male subjects (42 to 60 years)	Finland	17.7 years	Serum long-chain n-3 PUFAs	No association with EPA level RR 0.96 (95% CI; 0.64-1.42) p =0.70 Lower AF risk with DHA level RR 0.62 (95% CI; 0.42-0.92) p= 0.02
Cardiovascular Health study (Wu 47)	3,326 subjects (60% women) Mean age: 74.1 ± 5.2 years	USA	10 years	Serum long-chain n-3 PUFAs	Lower AF risk with total n-3 PUFAs level RR 0.64 (95% CI; 0.52-0.79) p < 0.001 Lower AF risk with DHA level RR 0.77 (95% CI; 0.62-0.96) p = 0.01

AF: atrial fibrillation; RR: relative risk; CI: confidential interval; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid

AF. However, the role of the direct measurement of n-3 PUFAs level in blood sample as marker of risk of AF development appears to be disputed by some studies that suggest a genetic predisposition in the incorporation of fatty acids in erythrocytes membranes.[48,49] In particular Viviani Anselmi and coworkers[49] evaluated the percentage of fatty acids in erythrocytes membranes of 40 patients with idiopathic AF and 53 age-matched healthy subjects. Their results showed significantly lower levels of monounsaturated and saturated fatty acids and significantly higher concentrations of PUFAs in the erythrocyte membranes of AF patients compared with the control group, in spite of similar dietary habits. The authors concluded that an imbalance between saturated, cis and trans unsaturated fatty acids could indicate a susceptibility to oxidative stress and arrhythmias.

N-3 PUFAs and Post-Operative Atrial Fibrillation

In an open-label, randomized trial for the primary prevention of post-operative AF, a significant reduction in the incidence of post-operative AF and a significant shorter hospital stay were observed in 160 patients treated with 2 g daily of n-3 PUFAs for at least 5 days before elective CABG and until the day of discharge from the hospital. N-3 PUFAs administration during hospitalization reduced the incidence of postoperative AF by 54.4% and was associated with a shorter hospital stay.[50] In agreement with these results, intravenous infusion of n-3 PUFAs at 100 mg/kg/day, at least 12 h preoperatively and immediately following surgery, was associated with a significantly lower incidence of post-operative AF as well as shorter hospitalization compared with placebo.[51] At odds with the results of the above trials, Saravanan and colleagues reported that, in 108 patients undergoing isolated CABG, no significant difference in the incidence of post-operative AF was found between patients treated with n-3 PUFAs and those on placebo, despite significantly higher serum and right atrial appendage levels of n-3 PUFAs measured in the treated group.[52] Similarly, Heidarsdottir and colleagues reported no evidence for a beneficial effect of treatment with N-3PUFAs on the occurrence of postoperative AF in patients undergoing open-heart surgery.[53] A recent meta-analysis including data from of all the above randomized trials shows that in aggregate n-3 PUFAs treatment is not associated with a reduction in the incidence of AF following cardiac surgery.[54] Differences in patient profiles, type of surgery, definition of arrhythmia and method of arrhythmia surveillance may explain the conflicting results of these studies (Table 2). For example, Heidarsdottir and colleagues used a Holter monitor worn from the immediate post-operative period throughout hospitalization to detect the occurrence of AF,[53] whereas in the other studies an ECG was performed during the hospitalization only if symptoms were present. This major methodological difference is the likely explanation of the lower incidence of AF in reported by these studies and may have underestimated the therapeutic effects of n-3 PUFAs treatment. Also the different period of n-3 PUFAs administration may explain the different results observed in these studies. Only Calo and coll[50] and Heidt and coll[51] administrated fish oil supplementation in the immediate post-operative period (through a nasogastric tube and intravenously respectively) improving delivery of n-3 PUFAs during this 'critical' period that may be important for successful prevention of postoperative AF. In fact, inflammation and oxidative stress, which have been recognized as pivotal mechanisms involved in the development of post-operative AF, are particularly intense in the earliest days after cardiac surgery.[5] The importance of inflammation in the immediate post-operative AF occurrence and the beneficial role of pre-operative n-PUFAs supplementation is emphasized in a study by Mariscalco and colleagues.[55] In this prospective observational study including 530 patients who had consecutively undergone isolated CABG, isolated valve procedure or combined procedures, the authors evaluated the influence of n-3 PUFAs therapy on early and late occurrence of AF. Postoperative AF occurring in the surgical department was defined as 'early AF,' whereas that occurring during cardiac rehabilitation program was classified as 'late AF'. The overall incidence of early AF in the whole study sample was 44.7%, while late AF occurred in 14.7% of the patients. On multivariable analysis, pre-operative n-3 PUFAs therapy was associated with a significant reduction (OR 0.54, 95% CI 0.31-0.92; $p < 0.05$) in early AF, but the same effect was not demonstrated for the occurrence of 'late AF' (OR 1.3, 95% CI 0.52-3.29).

Since inflammation and oxidative stress contrib-

ute to the risk of postoperative AF, Cereceda and colleagues tested the hypothesis that combined therapy with n-3 PUFAs and an antioxidant agent, such as vitamin C and E, may have synergistic effects on the risk of AF after cardiac surgery. In a preliminary study, the authors reported that the combined therapy reduced atrial tissue markers of oxidative stress and inflammation in patients undergoing on-pump cardiac surgery.[56] More importantly, in a dedicated investigation, supplementation with n-3 PUFAs plus vitamin C and E reduced the incidence of atrial fibrillation by 73%.[57]

The results of the recent "Fish oil to inhibit supraventricular arrhythmias after cardiac surgery: the FISH trial", currently published only in abstract form,[58] also appear to show no significant effects of n-3 PUFAs treatment in the incidence of postoperative AF. An important methodological feature may account, at least in part, for the negative results of this study. The majority of participants were taking beta-blocker or statin therapy (80% and 74%, respectively), which might have reduced the magnitude of the effects of n-3 PUFAs in this population.

Recently, Skuladottir and colleagues examined the association between plasma n-3 and n-6 PUFAs and the incidence of post-operative AF in 125 patients who took part in their previous study[53] and in whom plasma levels of fatty acids had been measured.[59] As there was no difference in the incidence of post-operative AF between the n-3 PUFAs and placebo group, the treatment assignment was ignored in this analysis. Results showed that patient who did develop post-operative AF had lower plasma levels of arachidonic acid ($p < 0.05$) and higher levels of DHA ($p < 0.05$) compared with patient who did not develop post-operative AF. Moreover for the post-operative total n-3 PUFAs levels the authors found a non significant U-shaped association with post-operative AF, suggesting that n-3 PUFAs supplementation after cardiac surgery may be beneficial only in patient with low pre-operative plasma fatty acids levels.

Farquharson and colleagues conducted a prospective, randomized, placebo controlled study to examine the effect of fish oil supplementation (started 3 weeks before the scheduled date for surgery) in patients undergoing CABG and/or valve replacement.[60] Plasma levels of n-3 PUFAs were determined at baseline and before cardiac surgery in all patients. Despite a significant increase in EPA and DHA plasma level in the treatment group at the time of surgery, pre-operative fish oil supplementation was not associated with a statistically significant decrease in time to a first AF event (HR = 0.66; 95% CI, 0.43-1.01; p = 0.06). The results of this study also showed a different incidence of AF between CABG and valve surgery group. A differential impact of the type of surgery on event rate has also been reported in a previous large multicenter study that enrolled patients undergoing different cardiac operations[61] suggesting different causal conditions for post-operative AF. In order to identify the impact of n-3 PUFAs therapy on the incidence of post-operative AF according to different CABG technique ('off pump' vs 'on pump'), Sorice and colleagues randomized 201 patients to receive 2 g/day of n-3 PUFAs or placebo from at least 5 days before surgery until hospital discharge. Post-operative AF occurred in 11.4% of the patients assigned to the treatment group and in 22.8% of the patients randomized to placebo (OR 0.43; 95% CI 0.2-0.95; p = 0.033). A significant reduction of post-operative AF incidence was observed only in patients treated with n-3 PUFAs undergoing "on pump" CABG.[62] Since CABG induces a systemic inflammatory response by triggering the production and release of inflammatory mediators, this finding may further support the hypothesis of the anti-inflammatory effects of n-3 PUFAs.

In summary, definitive evidence supporting the efficacy of n-3 PUFA supplementation in the reduction of the AF risk in patients undergoing cardiac surgery is still lacking. The results of the multicenter, multicountry "Omega-3 Fatty Acids for Prevention of Post- Operative Atrial Fibrillation" (OPERA) trial (ClinicalTrials.gov no. NCT00970489)[63] are expected to deliver a more definitive answer regarding the therapeutic role of n-3 PUFAs in the primary prevention of post-operative AF. Additionally, OPERA will help to elucidate the pathogenetic mechanisms of postoperative AF and improve our understanding of the antiarrhythmic effects of n3-PUFAs in AF.

N-3 PUFAs and Atrial Fibrillation after myocardial infarction

Atrial fibrillation is frequently observed in the

setting of acute myocardial infarction and the development of this arrhythmia is associated with an increased risk of death and stroke. Older age, left ventricular dysfunction and clinical congestive heart failure were found to be the most important independent predictors for the development of AF after myocardial infarction.[64,65,66] Based on the positive results obtained from the GISSI-Prevenzione,[67] the European Society of Cardiology in 2003 added to their guidelines n-3 PUFAs supplemen-

Table 2 **Effect of Omega-3 Polyunsaturated Fatty Acids Supplementation for the Primary Prevention of Postoperative Atrial Fibrillation. Clinical Trials**

First Author Ref	Trial Design	n	Treatment	Type of Surgery	Main Results (% of pts with post-op AF)		
					n-3 PUFAs	Control	P value
Calò [50]	Open label, no placebo	160	Oral 2g/day at least 5 days before surgery and until discharge	CABG	15.2%	33.3%	0.0013
Heidt [51]	Placebo controlled	102	Intravenous 100 mg/kg/day at least 12 h before surgery and until transfer to the war	CABG	17.3%	30.6%	< 0.05
Cerceda [57]	Placebo controlled	83	Oral 2 g/day plus vit C and E	CABG or valve surgery	AF incidence reduced by 73% in treatment group (preliminary results)		
Saravanan [52]	Double blind placebo controlled	108	Oral 2g/day at least 5 days before surgery and until discharge	CABG	29%	22%	0.28
Heidarsdottir [53]	Double blind placebo controlled	170	Oral 2g/day at least 5-7 days before surgery until 2 weeks after surgery	CABG or CABG and valve surgery	54.2%	54.1%	0.99
Sandesara [58]	Double blind placebo controlled	243	Oral 2g/day at least 3 days before surgery and until discharge	CABG or CABG and valve surgery	30%	33%	0.67
Farquharson [60]	Double blind placebo controlled	194	Fish oil 15 ml/day 3 weeks before surgery and 6 days after or until discharge	CABG and/or valve surgery	37%	48 %	0.11
Sorice [62]	Double blind placebo controlled	201	Oral 2 g/day five days before surgery and until discharge.	CABG (off pump and on pump technique	11.4%	22.8%	0.033

pts: patients; post-op AF: post-operative atrial fibrillation; n-3 PUFAs: omega-3 polyunsaturated fatty acids; CABG: coronary artery bypass grafting

tation at the dose of 1 g/day in patient with documented coronary artery disease.[68] A record-linkage analysis of a database of 3242 patients hospitalized with myocardial infarction in Italy from January 2002 to December 2004 showed that prescription of N-3 PUFAs supplements in 215 of them was associated with a reduction in relative risk of hospitalization (HR 0.19; 95% CI 0.05-0.46) for AF and in all cause mortality (HR 0.15; 95% CI 0.05-0.46).[69]

N-3 PUFAs and Secondary Prevention Studies

In a recent meta-analysis of five studies including a total of 1,179 patients, no significant effects of n-3 PUFAs supplementation on the recurrence of AF were observed (odds ratio 0.83, 95% confidence interval 0.48 to 1.45; P=0.51).[70] This meta-analysis included the results of our recently published double-blind, placebo controlled trial, which showed that n-3 PUFAs supplementation, in addition to a background therapy with amiodarone and an ACE-i or an ARB is more effective in maintaining sinus rhythm after cardioversion in patients with persistent AF than therapy with amiodarone and an ACE-i or an ARB alone. In particular, we found that the mean time to the first recurrence was significantly higher in patients on n-3 PUFAs than in placebo group (167.72+116.26 vs 139.21+112.63, respectively; p<0.001), with a rate of AF recurrences throughout 1-year follow-up significantly lower (at 12 months 39% vs 62.6%, respectively in the treatment and control patients; p<0.002).[71] Our results are at odds with those of others secondary prevention trials, but several methodological factors may explain, at least in part, these discordant findings (Table 3). In a study by Kowey and colleagues, which enrolled 663 patients with confirmed symptomatic paroxysmal (n=542) or persistent (n=121) AF, among patients with paroxysmal AF there was no difference between treatment groups (n-3 PUFAs or Placebo) for recurrence of symptomatic AF (HR 1.15; 95% CI, 0.90-1.46; P = 0.26).[72,73] Among patients with persistent AF the rate of arrhythmia recurrence, although not statistically significant, was smaller in the treatment group. In contrast to this study, we enrolled older patients, with persistent AF and almost all with structural remodeling. Also the recently published results of a prospective non-randomized study in 50 patients with paroxysmal AF and without associated structural heart disease showed that EPA supplementation did not have an effect on the number of AF paroxysms compared to the treatment with anti-arrhythmic drugs alone. All patients enrolled were initially treated with antiarrhythmic drugs (Propafenone or Flecainide) for 6 month, and thereafter EPA was added at a dose of 1800 mg/die. After 1 year of follow-up, no differences on AF recurrences were found between observational and interventional period.[74] The mechanism underlying the pathogenesis of AF may differ between paroxysmal and persistent form and the beneficial effects of n-3 PUFAs may become clinically evident only in patients with persistent AF and in the presence of atrial remodeling.[75,76] Also a trial conducted by Bianconi and colleagues failed to demonstrate a difference between n-3 PUFAs- and placebo-treated groups in the prevention of AF recurrence after electrical cardioversion.[77] It is important to note that, in the latter study, only 27.8% and 66.8% of patients were taking amiodarone and ACE-I/ARBs respectively, and that in Kowey's study[73] the use of amiodarone was not allowed and only 41% of patients were on ACE-I/ARBs therapy. Instead, in our study all participants were treated with both amiodarone and ACE-I/ARBs therapy, given the favorable results of the combined use of these two drugs in reducing atrial remodeling and AF recurrences.[78,79] Thus it is reasonable to speculate that the anti-arrhythmic effects of n-3 PUFAs could be complementary and synergistic with both membrane-active antiarrhythmic drugs, as well as with anti-remodeling agents. In a recent randomized, placebo controlled study, Kumar and colleagues found that fish oil supplementation (6 g/day) significantly reduced the AF recurrence after electrical cardioversion of persistent AF, independently from the concomitant antiarrhythmic therapy.[80] Another important methodological difference between all the studies described above is the duration of the n-3 PUFAs pre-treatment before direct current cardioversion (DDCV). Recently, Metcalf and colleagues examined the kinetics of incorporation of n-3 PUFAs into human atrial cell membrane removed during a standard surgical procedure. After supplementation with fish oil at different time before surgical intervention these authors found that the incorporation of n-3 PUFAs into phospholipid bilayer membrane is curvilinear and continues after achievement of stable plasma concentration, reaching a maximum at approximately 30 days of treatment.[81] Bianconi and colleagues[77] started

Table 3 | **Polyunsaturated Fatty Acids for the Secondary Prevention of Atrial Fibrillation. Clinical Trials**

First Author ref	n	Mean age	Clinical setting	Mean EF	Treatment	Follow up	Anti-Arrhythmic Drugs	Main Results
Nodari [71]	199	70 ± 6 (n-3 PUFAs) 69 ± 7.9 (placebo)	Post DCCV persistent AF	49 ± 11 % (n-3 PUFAs) 50 ± 10 (placebo)	2 g/day 4 weeks before DCCV and until the end of follow-up	12 month	100% Amiodarone 100% ACE-I/ARBs	Early recurrences: 6% vs. 12.1% (p < 0.01) Late recurrences 40% vs. 63% (p= 0.007)
Bianconi [77]	204	62.9 ± 7.9	Post DCCV persistent AF	57.7 ± 11.3%	3 g/day for 1 week before DCCV; 2 g/day until the end of follow-up	6 month	27.8% Amiodarone 66.8% ACE-I/ARBs	% of patients with AF recurrences 58.9 % vs. 51.1% (p=0.28)
Kowey [73]	663	58.2% ± 13.6	Paroxysmal AF (n= 542) or Persistent AF (n=121)	Not stated	8 g/day loading dose for 7 days; 4 g/day until the end of follow-up	6 month	Amiodarone not allowed at inclusion 40% ACE-I/ARBs	Paroxysmal group % symptomatic AF recurrences: 52% vs. 48% (p=0.26) Persistent group % symptomatic AF recurrences: 50% vs. 33% (p=0.09)
Patel [84]	285	60 ± 5.8 (n-3 PUFAs) 58 ± 11 (control group)	Post-ablation	54 ± 8 % (n-3 PUFAs) 53± 5 % (control group)	A minimum of 665 mg/day for 1 month before ablation and until the end of follow-up	28 ± 7 month	Not stated	Early recurrence: 27.1% vs. 44.1% (p< 0.0001) Late recurrence: 23.2% vs. 31.7% (p< 0.003)
Watanabe [74]	50	54 ± 9	Paroxysmal AF	Not stated* *All patients didn't have structural heart disease	Antiarrhythmic drugs for 6 month and thereafter EPA at a dose of 1.8 g/day for 6 month	1 year	60% Propafenone 40% Flecainide	No significant difference in the number of days of AF per month before and after intervention (2.6 ± 2.2 days/months vs. 2.5 ± 2.2 days/months; p = 0.45)
Kumar [80]	178	63 ± 10 (n-3 PUFAs) 61 ± 13 (control group)	Persistent AF	59.7 ± 10.3 (n-3 PUFAs) 57 ± 12.2 (control group)	6 g/day 4 weeks before DCCV and until the end of follow-up	12 month	33.3 % Amiodarone 44.8 % Sotalolo 21.8 % beta blockers, digoxin or Ca2+ antagonists	AF recurrences was significantly lower in patents receiving omega 3 than placebo (HR 0.35; 95% CI 0.24 – 0.51; p< 0.001)

EF: ejection fraction; DCCV: Direct Current Cardioversion; AF: atrial fibrillation; ACE-I: angiotensin-converting enzyme inhibitors; ARBs: angiotensin II receptor blockers

n-3 PUFAs treatment at least 1 week before electrical cardioversion and they found a high recurrence rate in the first weeks after cardioversion (mean time to first recurrence of AF was 83 ± 8 days in the PUFA treated group). In the study by Kowey and colleagues[73] in which participants received a loading dose of 8 g/day for 7 days and then maintenance dose of 4 g/day through follow up, nearly half of AF recurrences occurred within the first two weeks. In our study,[71] electrical cardioversion was performed at least four weeks after the beginning of n-3 PUFAs treatment and recurrence rates were lower in the first weeks (mean time to a first recurrence was 168 ± 116 in the treatment group). Also in the study by Kumar and colleagues, in which treatment with omega 3 was commenced > 1 month prior to electrical cardioversion, the mean time to the first AF recurrence was 190 days in the treatment group.[80] It is therefore reasonable to assume that the higher percentage of early AF relapses observed in these studies could be due to an inadequate pretreatment time for the incorporation of fatty acids in cell membranes rather than a lack of their efficacy. Another important difference with the study by Kowey and colleagues[73] is that they used a biweekly transtelephonic monitoring during follow up, while in our study[71] all patients with successful DCCV underwent weekly clinical and ECG controls for the first 3 weeks. Subsequently, follow-up visits with clinical evaluation, ECG, and a 24-hour Holter monitoring were scheduled at 1, 3, 6, and 12 months after DCCV. Moreover, other healthcare professionals operating in our anticoagulation clinic provided to notify us in case of incidental detection of AF relapses. The possibility to conduct a specialized follow up may allow a greater accuracy to detect asymptomatic episodes of AF relapses. A large on-going secondary prevention Fish Oil Research with omega 3 for Atrial Fibrillation Recurrences Delay (FORωARD) study (ClinicalTrials.gov no NCT00597220) is expected to provide further information on the effects of n-3 PUFAs supplementation for the secondary prevention of paroxysmal and persistent AF.[82]

In recent years, radiofrequency catheter ablation has emerged as a highly effective treatment strategy in patients with paroxysmal and chronic AF. However, AF may recur within days to weeks after a successful ablation procedure in up to 50% of the patients, probably because of an inflammatory response to the thermal injury caused by radiofrequency energy application.[83]In this setting, Patel and colleagues found that in a retrospective study of 258 patients undergoing PV ablation, the use of n-3 PUFAs supplementation was associated with a lower incidence of AF recurrence compared with non-users (23.2 vs. 31.7%; $P < 0.003$) and with a significantly larger reduction in CRP level (14.3 ± 2.1 vs. 18 ± 3.1 mg/L; $p = 0.0001$).[84] Despite the study limitations, these findings seem to suggest a possible positive role of n-3 PUFAs for the prevention of AF recurrences after pulmonary vein ablation.

Conclusions

The multiple antiarrhythmic properties of n-3 PUFAs have been well documented in several experimental studies. In particular, beyond their effects on ion membrane currents, n-3 PUFAs may favorably impact other pathogenetic arrhythmic substrates, such as structural and electrical remodeling, and the sympatho-vagal balance. In aggregate, this evidence supports a potentially beneficial role of n-3 PUFAs in the prevention and treatment of AF. Epidemiological and clinical studies for the primary and secondary prevention of AF have shown conflicting results, which can be accounted for, at least in part, by methodological differences or study limitations, such as different patient population, treatment dose, use of concomitant therapies, detection of AF, and timing of the follow-up. To date, there is insufficient evidence indicating that n-3 PUFAs treatment may be useful for the prevention of AF in patients undergoing cardiac surgery. There is also no robust evidence to make any recommendation for the use of n-3 PUFAs for the secondary prevention of AF. The results of ongoing trials will shed light on the current uncertainties. In addition, in our opinion, focus studies should be conducted with well-selected and homogeneous populations to address important but still unanswered questions regarding the most effective dose of n-3 PUFAs (is higher better?) and formulation (should DHA or EPA be used alone or in combination? What is the most effective ratio of EPA to DHA?) and to identify the patient population that may benefit the most from n-3 PUFAs supplementation.

Disclosures

No disclosures relevant to this article were made

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