REVIEW ARTICLE

Omega-3 PUFAs and Atrial Fibrillation: Have We Made Up Our Mind Yet?

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Atrial fibrillation (AF) is the most common type of arrhythmia in adults, accounting for about one third of total arrhythmia-related hospitalizations. AF impact on daily clinical practice is steadily rising, together with population aging and increased survival from underlying conditions closely associated with AF such as coronary heart disease and heart failure. Although antiarrhythmic therapy, oral anticoagulation, implanted device therapy, and ablation techniques are now all common and promptly available strategies in AF management, some of them are burdened by a low efficacy rate, while others are associated with increased proarrhythmic or hemorrhagic risk. Consequently, useful alternatives are being sought. Between those, polyunsaturated fatty acids (n-3 PUFAs) have risen from mere alternative to statins in dyslipidemia management to powerful and well-tolerated antiinflammatory, antithrombotic, and antiarrhythmogenic drugs.

From the evidence collected through basic science studies, whether on in vivo myocytes, animal models, or surrogate end points in human, n-3 PUFAs seem to offer innumerable advantages. On the other hand, epidemiological and clinical trials failed to demonstrate a clear efficacy of n-3 PUFAs as antiarrhythmic drugs, although covered by an optimal safety profile. The aim of the present review is to summarize the most important evidences currently available on the role of n-3 PUFA in AF management and therapy.

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atrial fibrillation; polyunsaturated fatty acids; antiarrhythmic drugs

Atrial fibrillation (AF) is the most common type of arrhythmia in adults, accounting for about one-third of total arrhythmia-related hospitalizations.¹ AF prevalence ranges from 0.4% to 1% in the general population and increases with age reaching 8% in patients older than 80 years.^{2–4} AF impact on daily clinical practice is steadily rising, together with population aging⁵ and increased survival from underlying conditions closely associated with AF such as coronary heart disease and heart failure.^{6,7} Although antiarrhythmic therapy, oral anticoagulation, implanted device therapy and ablation techniques are now all common and promptly available strategies in AF management, some of them are burdened by a low efficacy

rate, while others are associated with increased proarrhythmic or hemorrhagic risk. Consequently, useful alternatives are being sought. Between those, polyunsaturated fatty acids (n-3 PUFA) have risen from mere alternatives in dyslipidemia management to powerful and well-tolerated anti-inflammatory, antithrombotic, and antiarrhythmogenic drugs.^{8,9} Thus, these products have been studied in clinical trials involving treatment of AF in a wide range of patients and clinical scenarios.

The aim of the present review is to summarize the most important evidences currently available on the role of n-3 PUFA in AF management and therapy.

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EFFECTS OF n-3 PUFAs ON IN VITRO MYOCARDIOCYTES AND ANIMAL MODELS

The ability of dietary lipids to influence arrhythmia occurrence in animal model has been known for at least 25 years. In 1988, a study first reported that polyunsaturated alpha-linoleic acid lowered the arrhythmia threshold of the isolated rabbit heart.¹⁰

This finding led to a series of studies on the functional and antiarrhythmic actions of fatty acids on in vitro cultured, neonatal rat heart cells,^{11,12} and electrophysiological studies.¹³ The latter had shown that there are at least three possible levels of action for the PUFAs on the myocyte^{12,14} that may result in a significant protection against fibrillation: a slight hyperpolarization of the resting membrane potential, an increase of the current necessary to elicit an action potential, and an increase of the phase 4 refractory period. These effects were found to be related to an inhibition of the sodium current I_{Na} , of the calcium current I_{CaL} , and, possibly, of the potassium currents I_K and I_{to} .¹⁴

Li et al. showed that acute administration of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid in isolated human atrial myocytes inhibited transient outward (I_{to}), ultrarapid delayed rectifier potassium currents (I_{Kur}), and the voltage gated sodium current (I_{Na}) in a concentration-dependent manner.¹⁵ I_{to} and I_{Kur} are important for cellular repolarization, and their inhibition has been shown to prolong action potential duration in the human atrium, thus conferring an antifibrillatory effect.¹⁶ Moreover, n-3 PUFAs have been shown to modulate L-type calcium channels, reducing fluctuations in cytosolic calcium via the sarcoplasmic reticulum.^{11,12}

Leaf et al. confirmed these results, in fact they showed that PUFAs modulate ion currents in the myocyte sarcolemma, shifting the steady-state inactivation potential to more negative values, increasing the depolarizing current necessary to elicit an action potential by 50% and prolonging the refractory period by about threefold.¹⁷ Another antiarrhythmic effect of PUFAs is due to an increased activity of cardiac microsomal Ca^{2+}/Mg^{2+} -ATPase.¹⁸

All mentioned mechanisms seem to prevent atrial electrical remodeling, which is a well-known mechanism underlying AF perpetuation. Further

studies had been conducted on experimental models of AF.

Ninio and colleagues studied the effect of dietary fish oil on the rabbit model of stretch-induced vulnerability to AF.¹⁹ They found that total red blood cell count, atrial, and ventricular omega-3 fatty acid levels were significantly higher in the fish oil group than in control group and that lower pressures were needed to induce and sustain AF in the latter group. Moreover, they found that AF episode duration was shorter in rabbits with high PUFA content in atrial myocytes. On the basis of these results, they concluded that incorporation of dietary omega-3 fatty acids into atrial tissue reduces stretch-induced susceptibility to AF.

Sakabe et al. showed that PUFAs suppress congestive heart failure induced atrial structural remodeling and AF promotion but do not affect atrial tachycardia induced electrical remodeling.²⁰ In fact, they found that n-3 PUFAs had no effect on effective refractory periods or baseline conduction velocity. However, PUFAs supplementation provokes fewer slow conduction zones on myocytes, attenuates conduction heterogeneity, reduces atrial fibroses and duration of the AF episodes. The mechanism by which PUFAs prevent heart failure induced atrial structural remodeling and AF promotion is still unknown, but could be related to a reduction in fibrous tissue production by MAP kinases, ERK and P38 pathway modulation. In fact, Ramadeen demonstrated that n-3 PUFAs treated dogs had a marked reduction in atrial fibrosis with downregulation of hypertrophy and inflammation-related genes.²¹

However, oral supplementation of n-3 PUFAs does not seem to affect atrial refractory periods, as suggested by Laurent and colleagues who showed that supplementation with one gram per day of n-3 PUFAs reduced AF vulnerability in a canine model of atrial cardiomyopathy, by inducing less local slowing of conduction and conduction heterogeneity, together with a smaller increase in collagen turnover.²²

We can postulate that the effect on myocytes of acute fish-oil supplementation may differ vastly from that of chronic administration. Chronic intake results in membrane incorporation, which probably provokes change in membrane fluidity and regulation of many gene expressions. In contrast, acute administration of PUFAs influences mostly ion channels resulting in repolarization of

Figure 1. Incidence of inducible AF. *Incidence of no inducible AF. The remaining bar graphs represent the incidence of any inducible AF categorized by time. (Reproduced with permission from Ref.²⁴)

myocardial membrane and increased arrhythmic threshold.²³

EFFECTS OF n-3 PUFAs ON HUMAN ATRIAL ELECTROPHYSIOLOGY

EPA and DHA in fish oils have been shown to have antiarrhythmic effects in cellular and animal preparations. However there is a paucity of published information on the effects of chronic n-3 PUFA supplementation on human atrial electrophysiology, and as such their mechanism of action in humans is poorly understood.

In a single blinded prospective study, 61 patients without clinical AF or structural heart disease undergoing on an electrophysiology study (EPS) were recruited into a control group (n = 30) and a fish oil group (n = 31). The latter were prescribed 6 g/day of fish oil for ≥1 month before performing EPS.²⁴

The main finding of this study was that chronic supplementation of n-3 PUFAs provoked 8% and 14% increase of atrial and coronary sinus refractoriness at all pacing cycle lengths. Moreover, there was an attenuation of maximal coronary sinus, interatrial, intraatrial, and left atrial conduction delay with no effect on baseline coronary sinus, interatrial, intraatrial, or left atrial conduction time and sinus node function. Finally, it was shown that n-3 PUFAs significantly reduced AF inducibility (Fig. 1).

We can postulate that increased atrial refractoriness was a potential reason for reduced vulnerability to AF in PUFA group. In fact, prolongation in atrial refractoriness may inhibit

Figure 2. Pulmonary venous effective refractory periods in the fish oil group (gray bars) and the control group (black bars). (Reproduced with permission from Ref.²⁵)

reentry that predisposes to AF. However, it must be taken into account that this effect is observed in patients without clinical AF or structural heart disease. The heterogeneous nature of AF pathogenesis modulated by interaction between atrial remodeling related to AF itself, age-related changes and influence of underlying structural heart disease could explain why other trials failed to show similar results.

In a subsequent trial, the same group evaluated the effect of chronic n-3 PUFAs supplementation on pulmonary veins (PV) and left atrial electrophysiology in patients with paroxysmal AF.²⁵ In this study 36 patients were randomized at least 1 month before undergoing PV ablation to receive supplementation with 6 g/day of fish oil (n = 18) or no therapy (n = 18).

It was found that PV and left atrial refractory periods were significantly longer in fish oil group compared to controls (Fig. 2). As a consequence of shorter pulmonary venous refractory period in controls, AF was initiated at short coupling intervals during refractoriness testing in 70.6% of controls versus 33.3% of cases.

It can be hypothesized that patients taking fish oil supplements had decreased susceptibility to PV initiated AF. Moreover, cycle length of first induced AF in PVs was significantly longer in patients using fish oil compared to controls. The arrhythmogenic substrate for AF in the PV is probably based on the interaction between triggered activity and abnormal electrophysiologic substrate.^{26,27} The evidence that patients assuming fish oil had greater refractory periods and AF cycle length, suggests that PUFAs supplementation could help to stabilize the arrhythmogenic substrate in patients with AF. These data are consistent with others showing that chronic n-3 PUFAs supplementation decreases the frequency and

burden of AF in patients with implanted devices and decreases early and late AF recurrences after PV isolation.²⁸

Another study tested if chronic fish oil supplementation could reverse atrial mechanical stunning after conversion of atrial arrhythmias to sinus rhythm. This important phenomenon is implicated in risk of thromboembolic complications, failure of improvement in cardiac output and exercise tolerance, and increased risk of recurrence after restoration of AF or atrial flutter.²⁹ Abnormalities in calcium cycling, atrial myolysis, cellular dedifferentiation, and atrial fibrosis are thought to be responsible for the development of stunning.

Kumar and colleagues had also studied the effects of n-3 PUFAs supplementation on atrial stunning. This prospective, randomized, single-blinded study enrolled 49 patients undergoing cardioversion of persistent AF or atrial flutter to sinus rhythm. Patients randomized to active group were prescribed 6 g/day of fish oil for at least 1 month before the procedure. Among these patients 25 underwent external cardioversion (19 for AF, 6 for atrial flutter), 17 underwent internal cardioversion (15 for AF, 2 for atrial flutter), and 7 underwent radiofrequency ablation for persistent atrial flutter.³⁰

The study showed that patients supplemented with chronic n-3 PUFAs for a mean of 70 days before the procedure had a left atrial appendage emptying velocity significantly higher than control group. This result has been shown in patients with AF as well as in patients with atrial flutter. Moreover, these data were independent from cardioversion technique and duration of arrhythmia. Another important finding was that patients treated with PUFAs had a greater preservation of left atrial appendage emptying fraction than control group. In the latter group there was an increased grade of spontaneous echocardiographic contrast after reversion. Finally this trial showed a significant reduction in the incidence of left atrial stunning after conversion of AF or AFL to sinus rhythm (Fig. 3).

EPIDEMIOLOGICAL STUDIES

Current evidence from prospective studies and randomized clinical trials suggests that regular intake of long-chain n-3 PUFAs found in fish and fish oil (EPA and DHA) may have protective effects

Figure 3. Atrial mechanical stunning was significantly lower in the omega-3 group. AF = atrial fibrillation; AFL = atrial flutter; LAAEV = left atrial appendage emptying velocity. (Reproduced with permission from Ref.³⁰)

against cardiovascular disease and antiarrhythmic effects especially on AF. In a prospective cohort study among elderly adults, regular intake of tuna or other broiled or baked fish, but not fried fish, was associated with lower risk of AF development.³¹ A total of 4815 adults ≥ 65 years were included into the study design and 980 of those developed AF, during 12 years follow-up. Risk of AF was reduced by 24% in patients who consumed tuna/other fish from 1 to 3 times per month (HR = 0.76, 95% CI = 0.61 to 0.95, $P = 0.02$). Risk was also 30% lower in patients who consumed tuna/other fish from one to three times per week (HR = 0.70, 95% CI = 0.57 to 0.87, $P = 0.001$), and by 35% in patients who ate fish more than four times per week (HR = 0.65, 95% CI = 0.51 to 0.84, $P = 0.0001$). The association held true after adjustments for a variety of demographic, clinical, lifestyle, laboratory, and dietary characteristics, including preceding myocardial infarction and congestive heart failure. In contrast, fried fish/fish sandwich consumption was associated with higher risk of AF.

The authors observed that this relationship could be due to the effects of long-chain n-3 PUFAs present in fatty fish. This can explain why a lower risk of AF was not observed in association with the intake of fried fish or fish sandwiches, which are usually made from lean fish and did not correlate with n-3 PUFAs levels. Frying can alter greatly a fish meal's nutrient composition, increasing contents of n-6 fatty acids, trans-fatty

acids, and oxidation products, particularly when oils are repeatedly used for frying.

The Kuopio Ischemic Heart Disease Risk Factor Study was designed to investigate risk factors for cardiovascular disease, atherosclerosis and related outcomes in a randomly selected sample of men from eastern Finland. One of the main targets of this study was to test whether high serum concentrations of EPA, DHA, and docosapentaenoic acid, which also serve as a marker of fish or fish oil consumption, were associated with risk of incident AF in middle-aged or older men.³² A total of 2174 subjects were studied. During an average follow-up time of 17.7 years, 240 AF events occurred. Results were less encouraging than expected. If valuated individually, only serum DHA was associated with a reduced risk for AF. Exclusion of 233 subjects with myocardial infarction or congestive HF either at baseline or preceding AF episodes slightly strengthened the association. The main strength of the present study's methodology is the use of serum long-chain n-3 PUFA measurements instead of food-frequency questionnaires for dietary intakes for estimating the impact of these fatty acids. On the other hand, only AF events documented on hospital discharge were included in the analysis and fish intake may have changed during the 17 years follow-up.

Unfortunately, there are also some negative evidences regarding the role of n-3 PUFAs as useful in preventing AF. In a prospective cohort study based on a subpopulation of the Rotterdam study, intake of fish oil was not associated with a reduced onset of AF.³³ A total of 5184 subjects with mean age of 76 years and free from AF at baseline were studied. Dietary intake was assessed using a semiquantitative food-frequency questionnaire and incidence of AF was continuously monitored during follow-up. Dietary intake of fish oils in the third tertile compared with first was not associated with the risk of AF. This finding is in line with Danish, Diet, Cancer and Health study, a large prospective cohort study on 47,949 patients in which chronic oral intake of n-3 PUFAs did not reduce the risk of AF.³⁴ In this study, the risk of AF or atrial flutter did not change substantially between specific types of fish consumed or by specific method of food preparation. Authors also concluded that increased fish oil intake was associated with an older age and with hypertension, which are both risk factors for hospitalization and therefore could lead more often

to ECG recording and therefore to an increased probability of asymptomatic AF detection. These associations, along with the exclusion from the study of all patients with any kind of heart disease, could have biased the results.

CLINICAL TRIALS OF POSTOPERATIVE AF

Atrial fibrillation occurs frequently after cardiac surgery with a reported incidence between 10% and 65%,^{35,36} and mostly between 48 and 72 hours after surgery.³⁷ Postoperative AF has been shown to increase long-term mortality after adjustment for known associated risk factors.³⁸ The results of the studies that assessed the effect of n-3 PUFA on the incidence of postoperative AF are discordant. Saravanan and colleagues studied the effect of n-3 PUFA compared to placebo on a group of 108 patients undergoing CABG.³⁹ Exclusion criteria were previous or current history of any atrial arrhythmia, administration of class I and III antiarrhythmic drugs or previous 3 months fish oil supplements consumption. Consecutive patients were randomly assigned, in a double-blind fashion, to receive 2 g/day of a commercially available n-3 PUFA preparation providing 85–88% EPA-DHA (active treatment group) or 2 g/day olive oil (placebo group) along with standard care at least 5 days before surgery (median 16 days) up to discharge. Postoperative continuous ECG monitoring was performed for 5 days or until discharge. Both EPA and DHA serum concentrations increased significantly in the n-3 PUFA group and were higher at surgery in the n-3 PUFAs group than in the placebo group. More importantly, EPA and DHA in atrial tissue collected at surgery were higher in the n-3 PUFAs group than in the placebo group. Postoperative AF was observed in 43% of the placebo group and 56% of the n-3 PUFA group, although log-rank test showed no significant difference in the AF-free distribution between the two groups (Fig. 4)

There was no clinical difference between groups in any of the secondary outcomes (clinically recognized AF, AF burden, length of hospitalization and length of stay in the intensive care or high-dependency care unit). Even Heidarsdottir et al. did not find a beneficial effect of treatment with n-3 PUFA on the occurrence of postoperative AF in patients undergoing open heart surgery.⁴⁰ This

Figure 4. AF-free survival curve for active (n-3 PUFAs) and placebo groups. (Reproduced with permission from Ref.³⁹)

prospective, randomized, double-blinded, placebo-controlled trial was conducted in patients admitted for coronary artery bypass grafting and/or valvular repair surgery. The patients received either n-3 PUFA capsules, containing a daily dose of 1240 mg EPA and 1000 mg DHA, or olive oil capsules for 5–7 days prior to surgery and postoperatively until hospital discharge. The end point was the occurrence of postoperative AF, defined as a single episode lasting more than 5 minutes detected by continuous electrocardiographic monitoring. There was no difference in the incidence of postoperative AF between active and control groups (54.2 vs 54.1%, respectively), although the total concentration of n-3 PUFAs in plasma phospholipids in the active group increased significantly from baseline to surgery day. No differences in the incidence of postoperative AF were found even when the study cohort was divided into tertiles of n-3 PUFAs blood levels at baseline and at the day of surgery.

The results of these two studies are conflicting with those of Calò et al., who indeed found an association between n-3 PUFAs and reduced postoperative AF in 160 patients in need for bypass surgery.⁴¹ The primary end point (development of AF in the postoperative period) was reached in 27 patients of the control group (33.3%) and in 12 patients of the PUFA group (15.2%), and the difference was statistically significant. There was

no significant difference in the incidence of nonfatal postoperative complications, and postoperative mortality was similar in the PUFA-treated patients (1.3%) vs controls (2.5%). After CABG, the PUFAs patients were hospitalized for significantly fewer days than controls (7.3 vs $8.2 \pm$ days).

These three trials offer conflicting evidences regarding usefulness of n-3 PUFAs in clinical prevention of postoperative AF. The populations studied, albeit quite similar between each other, were small in size and, regarding the two negative trials, the sample did not include a sufficient number of patients to rule out a possible correlation between n-3 PUFAs assumption and reduced postoperative AF. A bigger trial with a prespecified sample size and a focused primary end point is still needed.

CLINICAL TRIAL ON PAROXYSMAL AND PERSISTENT AF

The first available evidences regarding a possible role of n-3 PUFAs in nonpermanent AF management had shown some promising results. Macchia et al. assessed the effect of n-3 PUFAs on 1 year AF incidence in 208 patients hospitalized with a diagnosis of myocardial infarction.⁴² n-3 PUFAs reduced the relative risk of the hospitalization for AF by 19% over 1 year and were associated with a further and complementary reduction in all-cause mortality. Unfortunately, more recent data failed to confirm the impact of this therapy on AF prevention and progression. An American multicenter trial involving 663 outpatients with confirmed symptomatic paroxysmal ($n = 542$) or persistent ($n = 121$) AF and no substantial structural heart disease did not find differences between n-3 PUFA oral chronic treatment and placebo.⁴³ This negative result was achieved despite the fact that daily n-3 PUFA intake was indeed quite high in active group: 8 grams per day for the first 7 days and 4 grams per day thereafter for at least 6 months. Each 1 g capsule contained approximately 465 mg of EPA and 375 mg of DHA. There was no difference between treatment groups for recurrence of symptomatic episodes both in paroxysmal as well in persistent AF.

These results have been confirmed by Bianconi and colleagues on an European population.⁴⁴ In this multicenter trial 204 patients with persistent AF were randomly assigned to receive either 3 g/day

of n-3 PUFAs until external cardioversion for sinus rhythm restoration and 2 g/day n-3 PUFAs after or placebo. The cardiac rhythm after cardioversion was assessed by both trans-telephonic monitoring and clinical visits. Sinus rhythm was restored, either spontaneously or after external cardioversion, in 91.4% of the patients under PUFAs treatment and in 92.0% of the patients under placebo. AF relapses were also similar between the two groups (58.9% in the active group and 51.1% in the control group). Again, sample size was too small to exclude with certainty any possible advantage of n-3 PUFAs.

CONCLUSIONS AND FUTURE PERSPECTIVES

From the evidence collected through basic science studies, whether on in vivo myocytes, animal models or surrogate end points in human, n-3 PUFAs seem to offer innumerable advantages. It has been demonstrated that n-3 PUFAs produce a slight hyperpolarization of the resting membrane potential, increase the current necessary to elicit an action potential, prolong action potential duration in the human atrium, reduce inflammatory and remodeling molecular pathways, and reverse atrial stunning after cardioversion. So why have clinical trials been so inconclusive and contrasting about a real efficacy in everyday clinical practice? There are many possible explanations, but the most solid are based on a plethora of confounding factors which could explain the large discrepancies found between different results.

First of all, the biggest evidences in terms of raw number of patients come from the epidemiological studies. Unfortunately, most of them based their primary analysis on semiquantitative food-frequency questionnaire and not on actual serum levels of EPA or DHA,^{31,33} thus greatly limiting the chance to find a possible interaction between n-3 PUFAs and AF prevention. Moreover, questionnaires were often collected only at the beginning of the study, not taking into account all the changes in diet and lifestyle behavior that could have happened during the follow-up.

On the other hand, available clinical trials often enrolled only small groups of patients, and the statistical power is usually inadequate to exclude type 2 errors. Furthermore, clinical and demographical characteristics of the different

populations are very different from one another, making a comparison unfeasible. Regarding postoperative AF, for example, one study had enrolled only coronary artery bypass graft surgery patients,⁴¹ while another studied both bypass and valvular surgery patients.⁴⁰ Some clinical trials were performed only on AF patients without any kind of concomitant heart disease,⁴³ while others focused on ischemic heart disease⁴¹ or heart failure.²⁰ Finally, some studies tested n-3 PUFAs in addition to the standard of care for antiarrhythmic therapy⁴⁴ while others excluded patients already under treatment with any antiarrhythmic drug.^{42,43}

Another important question to address is the correct dosage of n-3 PUFAs to test in a clinical setting. At present date, we lack a single phase II, dose-ranging trial, and all the phase III trials available used a lot of different dosages and EPA/DHA ratios, without any solid reason to prefer a formulation over another. Moreover, an n-3 PUFAs intake of 8 grams per day did not show any net benefit in AF prevention,⁴³ while the much lower dose of 2 gram per day held significant results.⁴²

Finally, in most of the trial discussed, AF relapse has been defined only as clinical diagnosed AF through electrocardiogram or Holter monitoring. This strategy, albeit practical and easy to implement, does not take into account all the asymptomatic episodes of AF which did not require hospitalization but still provide a high risk for thromboembolic events and cardiovascular mortality.

In conclusion, nowadays there are no sufficient data to encourage n-3 PUFAs therapy in AF patients. A single dose ranging phase II trial is mandatory to establish the optimal dose. Only then, a big phase III trial with good statistical power, a hard primary end point and real-life population would allow us to make up our mind regarding this interesting issue.

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