Clinical Prevention of Sudden Cardiac Death by n-3 Polyunsaturated Fatty Acids and Mechanism of Prevention of Arrhythmias by n-3 Fish Oils

Alexander Leaf, MD; Jing X. Kang, MD, PhD; Yong-Fu Xiao, MD, PhD; George E. Billman, PhD

This review will be limited specifically to the beneficial prevention by the n-3 polyunsaturated fatty acids (PUFAs) of arrhythmic deaths, including sudden cardiac death, which annually causes some 300,000 deaths in the United States and millions more worldwide. We will also show that the growing body of positive clinical studies is supported by what has been learned in animal and laboratory studies regarding the mechanism by which n-3 PUFAs prevent cardiac arrhythmias.

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The Essential PUFAs

Figure 1 shows the 2 essential classes of PUFAs, the n-6 (ω6) and n-3 (ω3) classes. Both classes are “essential” because we cannot make them in our bodies. They must come in our diets, and they are essential for normal growth, development, and optimal function of brain, heart, and probably other systems. The parent fatty acid of the n-6 class, linoleic acid (C18:2n-6; LA) has 18 carbon atoms in its acyl chain, and the first C=C double bond is 6 carbons back from the methyl end of the fatty acid, hence the “n-6” appellation. In the bodies of animals, including humans, LA can be elongated and desaturated through a series of enzymatic steps to form arachidonic acid (C20:4n-6; AA). AA is the source of the n-6 eicosanoids that result from oxygenation of AA by cyclooxygenase, lipoxygenase, and epoxygenase enzymes to form prostaglandins, leukotrienes, lipoxines, and P-450 compounds, which in many instances are potent cell messengers.

In the chloroplast of green plants, algae, and phytoplankton, LA can be further desaturated in the n-3 position to yield α-linolenic acid (C18:3 n-3; ALA), the 18-carbon parent fatty acid of the n-3 class. ALA can be further elongated and desaturated by the same enzymes that convert n-6 LA to AA to form the 20-carbon n-3 analog of AA, namely, eicosapentaenoic acid (C20:5n-3; EPA). EPA in turn can compete with AA for the same cyclooxygenase, lipoxygenase, and epoxygenase enzymes to form a different class of eicosanoids, which in several important instances can oppose and counteract the action of the n-6 eicosanoids. The final elongation desaturation product of the n-3 class is docosahexaenoic acid (C22:6n-3; DHA), the longest and most unsaturated fatty acid normally encountered in our diets. EPA and DHA are physiologically the most important members of the n-3 class. Their source is largely from marine vertebrates, and they are accumulated in the phospholipids in our cell membranes, especially in brain, heart, and testes.

Clinical n-3 Fatty Acid Studies

The first clinical cardiovascular trial was reported in 1989 by Burr and associates. They performed a randomized controlled trial with a factorial design in 2033 men to determine whether dietary advice on fat, fish, or fiber is beneficial in the secondary prevention of myocardial infarction (MI). No benefits accrued from the fat and fiber advice. At the end of 2 years, however, there was a 29% reduction in mortality in the 1015 men who had received advice to eat oily fish, at least 200- to 400-g portions twice weekly, compared with the 1018 men who had not received such advice. There were no significant differences in ischemic heart disease events between the 2 groups because more nonfatal infarcts occurred in the group that was advised to eat fish. The mortality difference in favor of fish advice appeared early and persisted up to 2 years. Over the 2 years, there were no significant differences in levels of total cholesterol. This study was published before the antiarrhythmic effects of fish oils were generally known, but the reduced mortality in those advised to eat fish despite an increase in MIs in this cohort suggests that the findings resulted from a reduction in arrhythmic deaths. Fifty percent to 60% of deaths in the setting of coronary heart disease are sudden cardiac death (deaths within 1 hour of symptoms of an acute MI) attributed to sustained ventricular arrhythmias. Patients were still experiencing MIs but were not dying as frequently from them. The cardiology and medical communities paid little attention to this Diet and Reinfarction Trial (DART), coming as it did seemingly out of the blue.

The second noteworthy trial was published in 1994. It was a prospective secondary, single-blinded prevention trial comparing the effect of a Mediterranean ALA-rich diet (n=302) to the usual postinfarction prudent diet (n=303). The experimental group consumed significantly less lipids, saturated...
Polyunsaturated Fatty Acids

**n-6 Class**

- C18:2n-6 Linoleic
- C20:4n-6 Arachidonic
- C22:5n-6 Docosapentaenoic
- C22:6n-3 Docosahexaenoic

**n-3 Class**

- C18:3n-3 α-Linolenic
- C20:5n-3 Eicosapentaenoic
- C22:5n-6 Docosapentaenoic
- C22:6n-3 Docosahexaenoic

*Figure 1. Two classes of essential PUFAs.*

Fat, cholesterol, and linoleic acid but more oleic acids and ALAs, which was confirmed by measurements in plasma. The study was expected to last for 5 years, but after a mean follow-up of 27 months, the study was terminated by its Science and Ethics Review Committee because the results were so beneficial. There were already 16 cardiac deaths in the control group and 3 in the experimental group (of the 16 deaths in the control group, 8 resulted from sudden cardiac death, whereas there were no such deaths in the experimental group). Seventeen nonfatal MIs occurred in the control group and 5 in the experimental group, giving a combined risk ratio of 0.27 ($P=0.001$). Overall mortality was 20 in the control and 8 in the experimental group. Such clinical cardiac benefits have not been achieved before or since from solely a dietary study, and the trial requires confirmation. Benefits cannot be ascribed to the n-3 fatty acids specifically, although higher concentrations of n-3 fatty acids in plasma were documented in the experimental group. There were, however, considerable other dietary differences in intake of fresh fruits, vegetables, legumes, and grains. The benefits of the experimental diet appeared early and persisted for the full 2 years. There were no changes in blood cholesterol or in any component of the blood lipids. Despite termination of the study at 27 months and the requirement that each subject in the control group be informed personally of the reason that the study had been terminated, de Lorgeril and associates continued to follow both groups to 46 months’ follow-up and published their findings.

Surprisingly, the 70% mortality difference favoring the experimental group persisted. This speaks volumes for the palatability levels of the n-3 fish oil fatty acids were in the lowest quartile, the relative risk of sudden death was significantly lower among men in the third quartile (relative risk ratio, 0.28) and in the fourth quartile (0.19). The 72% and 81% relative risk reductions are the largest beneficial cardiac effects of the n-3 PUFAs reported thus far in humans.

More recently, the data in the Physicians’ Health Study have been examined to test whether n-3 fatty acid consumption would reduce the risk of sudden death in subjects without a history of preexisting cardiovascular disease. A prospective, nested, case-control analysis among apparently healthy men who were followed up for 17 years in the Physicians’ Health Study was conducted retrospectively.

The evidence has been strengthened that fish oil fatty acids can prevent sudden cardiac death in humans, and this may prove to be their major cardiac benefit. Indeed, in the DART and the GISSI-Prevenzione studies cited above, there were no reductions in nonfatal MIs.

**Animal and Laboratory Studies**

In the mid-1970s, Gudbjarnason and Hallgrimsson suggested that fish oils might be antiarrhythmic. Later, Murnaghan reported that ALA administered to a rabbit
heart in a Langendorff preparation increased the arrhythmia threshold of the heart. Two Australian investigators, Peter McLennan and John Charnock, began to study possible antiarrhythmic effects of the fish oil fatty acids.14 Their basic experiment was direct and simple. They fed rats diets in which they could control the major fat component for 3 or more months. At the end of the dietary period, they ligated the coronary arteries of the rats and counted the number of animals that died of sustained ventricular fibrillation (VF). In one report,15 McLennan showed that slightly more than 40% of the animals fed a diet with saturated fat providing 12% of energy calories died of sustained VF. This was not significantly reduced by an olive oil (monounsaturated fat) diet, whereas the group receiving tuna fish oil (rich in n-3 PUFAs) had no arrhythmic deaths. These investigators also reported a similar antiarrhythmic action of the n-3 PUFAs in nonhuman primates.16 Since then, others have repeated their findings in rats.17–20 When we learned about their findings, we decided that we should at least try to confirm their surprising results. This was done with a highly reliable dog model of sudden cardiac death.21 A surgically induced anterior MI was produced, and a hydraulic, inflatable cuff was left around the left circumflex coronary artery so it could be compressed at will. The dogs were then trained to run on a treadmill during the month allowed for recovery from the surgery and the anterior MI. The result of the exercise stress combined with an additional ischemic insult to the heart when the left circumflex artery was occluded caused some 60% of the dogs to go into a fatal VF within 2 minutes of the compression of the circumflex coronary artery. These responsive dogs provided a stable preparation in which to test potential antiarrhythmic agents and are the ones we studied. Figure 2 illustrates the typical response of one of the susceptible dogs to the exercise-ischemia protocol.22 The top control No. 1 tracing is an electroventriculogram. Because the dog was running on the treadmill, its pulse rate is elevated. The additional ischemic stress from occlusion of the left circumflex coronary artery for 2 minutes resulted in a ventricular flutter, and the circulation failed. As soon as the dog lost consciousness, it was defibrillated. The second tracing is of the same dog brought back into the laboratory 1 week later, when the same protocol was repeated. This time, just before the left circumflex coronary artery was occluded, an emulsion of free n-3 fatty acids was administered intravenously. Additional ischemic stress from occluding the left circumflex coronary artery this time failed to induce VF. One week later, control No. 2 was performed on the same dog, and an emulsion of soybean oil, which lacks any free n-3 fatty acids, was infused. Within 2 minutes of occluding the left circumflex artery, VF occurred. This is the protocol we used to test the antiarrhythmic action of the n-3 fish oil fatty acids with a control 1 week before and 1 week after the test with the infusion intravenously of the fish oil free fatty acids.22–24 The Table summarizes our experiments on the dogs. The prevention of fatal arrhythmias by the emulsion of fish oil concentrate (P<0.005) confirms the studies of McLennan et al.14–16 They used long feeding experiments, which were criticized because of possible confounding factors occurring in long-term dietary studies in animals. We intravenously infused free fatty acids just before an ischemic stress in our prepared dogs. We believed that if the n-3 fatty acid infusion

<table>
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<th>No. of Dogs Tested</th>
<th>Protected</th>
<th>P</th>
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<tr>
<td>Fish oil concentrate*</td>
<td>13</td>
<td>10</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>EPA†</td>
<td>7</td>
<td>5</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>DHA‡</td>
<td>8</td>
<td>6</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>α-LNA§</td>
<td>8</td>
<td>6</td>
<td>&lt;0.004</td>
</tr>
</tbody>
</table>

*72% n-3 PUFA with free EPA 33.9% and DHA 25%.
†98.4% free EPA; 1.1% free DHA.
‡90.8% free DHA; 0.9% free EPA.
§>99% free ALA.
were promptly associated with an effect in the protocol we used, we could feel confident that the effect resulted from what had just been infused.

Because fish oil contains many ingredients, we wanted to know which ingredients were effective. When we tested pure EPA and DHA, each alone carried on serum albumin was highly protective. Even the parent fatty acid of the n-3 class of PUFAs, ALA, which is not generally present in fish oil, was protective. We were unable to test sufficient dogs to learn whether one fatty acid was more potent than the other.

**n-3 PUFAs and Cultured Neonatal Rat Cardiomyocytes**

To learn whether there were any plausible biochemical or physiological effects of these n-3 fatty acids that could explain their antiarrhythmic action, the effects of the n-3 PUFAs on cultured neonatal cardiomyocytes were studied. One can quickly remove the hearts from several 1- to 2-day-old rat pups and separate the individual myocytes enzymatically. The myocytes are then plated on microscope coverslips and grown in appropriate culture medium. By the second day in culture, the heart cells are adherent in monolayer clumps to the coverslip, and each clump is beating spontaneously, rhythmically, and simultaneously. A coverslip with cells is transferred to a temperature-controlled perfusion chamber on an inverted microscope. With a video camera, a monitor screen, and an edge monitor, one can focus on the contraction of a single myocyte in a clump of myocytes and preserve a tracing of the contraction amplitude and rate of contractions with a recorder.

Figure 3 shows the characteristic slowing of the beating rate of the myocytes when low micromolar concentrations of EPA or DHA were added to the medium bathing the isolated heart cells. This effect was reversible. When delipidated bovine serum albumin (BSA) was added to the superfusate, the EPA or DHA was extracted from the heart cells, and the beating rate returned to the control rates. Toxic agents known to produce fatal arrhythmias in humans were added to the medium bathing the cultured cells, and the effects of adding the n-3 fatty acids were observed. We tested increased extracellular Ca²⁺, the cardiac glycoside ouabain, isoproterenol, lysophosphatidylcholine and acylcarnitine, thromboxane, and even the Ca²⁺ ionophore A23187. All of these agents induced tachyarrhythmias in the isolated myocytes.

Figure 4 shows the effects of elevated perfusate Ca²⁺ and ouabain on the myocytes. Both agents induced rapid contractions, contractures, and fibrillation of the myocytes. When

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**Figure 3.** Effects of EPA and DHA on contractions of isolated neonatal rat cardiomyocytes. Perfusion of myocytes with 5 μmol/L EPA (A) or 5 μmol/L DHA (B) reduced beating rate by 50% within 2 minutes. Addition of delipidated BSA to perfusate extracted EPA or DHA and returned contraction rate to control level. Tracing C shows similar effect of lidocaine (20 μmol/L) on contractions of cardiomyocytes.

**Figure 4.** Prevention and termination of arrhythmias by EPA. Perfusion of myocytes with medium containing 7 mmol/L Ca²⁺ (A, a) or 0.1 mmol/L ouabain (B, a) induced contraction and fibrillation of myocytes. Washing cells with medium containing Ca²⁺ (1.2 mmol/L) returned fibrillations to original beating rate (not shown). Myocytes were then perfused with medium containing 7 μmol/L EPA. When beating rate had slowed (5 to 8 bpm), addition of 7 mmol/L Ca²⁺ (A, b) or 0.1 mmol/L ouabain (B, b) failed to induce contractures or fibrillations. Slow beating rates were returned to original rates by perfusion with albumin (not shown). C, Fibrillation was induced by ouabain (0.1 mmol/L) plus Ca²⁺ (5 mmol/L) in perfusing medium. Addition of EPA (8 μmol/L) terminated fibrillation. Subsequent addition of delipidated BSA 2 mg/mL still in presence of ouabain and high Ca²⁺ concentrations, extracted free EPA, and fibrillation resumed.
EPA was added to the superfusate, the beating rate slowed, and when the high Ca\(^{2+}\) or ouabain was added in the presence of the EPA, no arrhythmia was induced. Furthermore, as shown in Figure 4C, after a violent fibrillation was induced in the cells by both elevated calcium and ouabain, addition of EPA stopped the arrhythmias, and the cells resumed their fairly regular contractions. The addition of the delipidated BSA to remove the free fatty acid from the myocytes resulted in recurrence of the arrhythmia. This taught us 2 important facts. First, the EPA could be extracted from the cells in the continued presence of the toxins, and the arrhythmia would return, which indicated that the fatty acids were acting without strong ionic or covalent binding to any constituent in the cell membrane. If they had such binding, we would not have been able to extract the EPA from the cells with the albumin. It appears the free fatty acids act directly on the heart cells and need only partition (dissolve) into the hospitable hydrophobic interior of phospholipids of the plasma membranes of myocytes to elicit their antiarrhythmic actions. Second, when we tested the ethyl ester of the EPA, it had no prompt antiarrhythmic action; only the free fatty acid with its negative carboxyl charge was antiarrhythmic.

At this point, we had found that the arrhythmias induced in the isolated neonatal rat cardiomyocytes could be prevented in every instance by the prior addition of EPA or DHA to the superfusate bathing the cells. Addition of EPA or DHA after an arrhythmia was induced would stop the arrhythmia. It was apparent that the n-3 PUFAs were affecting the excitability/automaticity of the cardiomyocytes, so the effects of the n-3 PUFAs on the electro-physiology of the myocytes were examined.\(^{29}\)

Heart, brain, and muscle are excitable tissues, and their function is to generate electrical currents to signal their actions in the body. They do this by activating and then inactivating ion channels in their plasma membranes to allow specific ions to move through their plasma membranes, thus creating ionic currents. In heart cells, these ionic currents create action potentials by the sequential opening and closing of fast voltage-dependent sodium currents into the cardiomyocytes (Figure 5). The fast movement of positive Na\(^{+}\) ions into the myocyte depolarizes the resting membrane potential and initiates an action potential. This is followed by outwardly directed potassium currents (\(I_{\text{K}}\), the initial outward current, and \(I_{\text{r}}\), the delayed rectifier current), which move positive K\(^{+}\) ions out of the cells repolarizing the myocytes back to their resting membrane potential. An inward calcium current, \(I_{\text{Cali}}\) temporarily delays repolarization of the membrane potential by bringing positive Ca\(^{2+}\) into the myocytes producing the plateau of the action potential. The orderly occurrence of these currents creates the action potentials, which couple the electrical and mechanical functions of the heart, resulting in its rhythmic contractions. Fatal arrhythmias occur when the electrical signals become chaotic and the heart can no longer function as a pump.

The n-3 fatty acids modulate the ionic currents in the plasma membrane of heart cells\(^{30,31}\) and the human myocardial sodium channel expressed in HEK293 cells.\(^{31,32}\) There is an effect of n-3 PUFAs on the sodium current, which contributes significantly to their antiarrhythmic action. These fatty acids shift the steady state inactivation to hyperpolarized potentials\(^{30–32}\) (Figure 6). When an ion channel opens, it is considered to be in its activated state. The subsequent closing of the ion channel occurs during its inactivated state. After an action potential, repolarization of the normal myocyte resting potential occurs promptly but before most sodium channels have recovered to their closed state, from which they can respond again with an action potential to another depolarizing stimulus. They are still relatively refractory, but that refractory period can be markedly prolonged by the presence of the n-3 fatty acids,\(^{31,32}\) which shift the steady state inactivation to hyperpolarized potentials. This simply means that in the presence of the n-3 PUFA, a considerably longer time or a more negative membrane potential is required to return the sodium channels to their resting, closed, but activatable state.

**Our Current Hypothesis**

Our current hypothesis regarding the mechanism of action of the n-3 PUFAs to prevent fatal arrhythmias is based on their actions to inhibit the fast, voltage-dependent sodium current\(^{30–32}\) and the L-type calcium currents.\(^{33}\) With an MI, a gradient of depolarization of cardiomyocytes occurs in the central core of the ischemic zone, cells rapidly depolarize and die. The depolarization results from deficiency of ATP in the ischemic cells, which causes a dysfunctional Na,K-ATPase and the rise of interstitial K\(^{+}\) concentrations in the ischemic zone. However, at the periphery of the ischemic zone, myocytes may be only partially depolarized. They become hyperexcitable because their resting membrane has become more positive, approaching the threshold for generating action potentials (activating fast Na\(^{+}\) channels). Thus, any additional small depolarizing stimulus (eg, current of injury) may elicit an action potential, which, if it occurs at a vulnerable moment during the cardiac electrical cycle, may initiate an arrhythmia. With nonhomogeneous rates of conduction pathways in the ischemic tissue, reentry arrhythmias are likely. In the presence of the n-3 PUFAs, however, a voltage-dependent shift of the steady state inactivation curve to more hyperpolarized potentials occurs. The consequence of this hyperpolarizing shift is that sodium channel availability is decreased, and the potential necessary to return these Na\(^{+}\) channels in partially depolarized myocytes to a closed but activatable state is physiologically unobtainable. Also, these

![Image of normal cardiac action potential](http://circ.ahajournals.org/content/circres/100/11/2650/F4.large.jpg)
partially depolarized cells have Na\textsuperscript{+}/H\textsuperscript{+} channels, which in milliseconds can slip into "resting inactivation" in response to subthreshold depolarizations without eliciting an action potential,\textsuperscript{34,35} and they do this even faster in the presence of the fish oil fatty acids.\textsuperscript{31,32} The results of these effects of the n-3 PUFAs is that these partially depolarized myocytes are quickly made inexcitable, and their potential arrhythmic mischief is aborted. Myocytes with normal membrane potentials in the nonischemic myocardium will not be so drastically affected by the PUFAs and will continue to function normally. In our opinion, this effect of the n-3 PUFAs on Na\textsuperscript{+}/H\textsuperscript{+} channels and their effect to inhibit L-type Ca\textsuperscript{2+} channels\textsuperscript{33} and prevent triggered arrhythmic afterpotential discharges caused by excessive cytosolic Ca\textsuperscript{2+} fluctuations are the major mechanisms for the antiarrhythmic effects of these PUFAs.

This electrical stabilizing action of the n-3 fatty acids can be demonstrated by a simple experiment. Figure 7 shows a continuous tracing of the contraction of a single myocyte in a clump of myocytes on a microscope coverslip.\textsuperscript{27} Initially, the myocyte is contracting regularly. Two platinum electrodes were placed across the coverslip, with their tips dipped into the fluid perfusing the heart cells, and connected to a voltage source. At 15 V, it was possible to double or triple the spontaneous beating rate. When the external voltage source was turned off, the cell resumed its control spontaneous beating rate. The next line is a continuation of the recording from the same cell. When EPA was added to the superfusate, the beating rate began to slow, and now the myocyte paid no attention to stimuli at 15 V or at 20 V from the external source. At 25 V, the cell responded, but only to every other stimulus. When delipidated BSA was added to the medium perfusing the myocytes, the EPA was extracted from the myocytes, and the beating returned to its control rate.\textsuperscript{27} This action of the n-3 fatty acids, which occurred directly on every cardiomyocyte on the coverslip in the absence of hormonal or neural control, indicates the potent electrical stabilizing action of these interesting n-3 fish oil fatty acids.

It should be mention that we have reported that electrical activity in brain neurons is similarly modulated by the n-3 fatty acids\textsuperscript{36,37} just as in the heart, also with potential health benefits. It is clear that that these long-chain PUFAs exert a basic control on all excitable tissues in our bodies: the cardiac, nervous, and probably skeletal muscular systems, which has not been generally recognized.

**Some Practical Aspects of the Clinical Cardiovascular Applications of n-3 Fish Oil Fatty Acids**

These n-3 fatty acids have been part of the human diet for some 2 to 4 million years, during which our genes were
adapting to our environment, including our diets. They are safe and have been listed on the GRAS (“generally regarded as safe”) list according to the Food and Drug Administration in amounts up to 3.5 g of fish oil per day.

With regard to how much of these essential n-3 fatty acids we should be ingesting, the recent advice of the American Heart Association that everyone should have at least 2 meals of oily fish per week appears to be a prudent general recommendation. Those with a family history of coronary heart disease or those who have a personal history of coronary heart disease should add a supplement of fish oil of some 600 mg of EPA plus DHA per day to be prudent. If there is a family history of sudden cardiac death, then the supplement should be increased to 1 to 2 g of EPA plus DHA. These are our personal recommendations, which have not been adopted by a government agency.

In conclusion, we have summarized the growing clinical evidence that these n-3 fatty acids are antiarrhythmic and can prevent sudden cardiac death in humans. We have also summarized studies on the mechanism of their antiarrhythmic action by modulating ion channels so as to stabilize the cardiomyocytes electrically.

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References


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