

# Antiarrhythmic effects of omega-3 fatty acids: From epidemiology to bedside

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Omega-3 polyunsaturated fatty acids are emerging as a safe and effective means to reduce sudden death after acute myocardial infarction. This review summarizes the epidemiological background for the use of omega-3 fatty acids with this indication, clinical trials performed so far, and experimental data supporting their antiarrhythmic efficacy. (*Am Heart J* 2003;146:420–30.)

Omega-3 (polyunsaturated) fatty acids (FA) are now under cardiologists' attention as potential antiarrhythmic agents. In the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto (GISSI)-Prevenzione Study, omega-3 FA significantly reduced the primary combined efficacy end point of death, nonfatal myocardial infarction, and nonfatal stroke, and also the relative risk of death. Such a decrease in mortality derived from a 20% reduction in total deaths, 30% reduction in cardiovascular deaths, and 45% reduction in sudden deaths.<sup>1</sup> No matter how striking they appeared, these results were not unexpected by scientists investigating the health benefits of omega-3 FA. A concordance of basic mechanistic studies, studies in animal models in vitro and in vivo, and clinical findings now consistently suggest that these substances may be effective and safe antiarrhythmic agents. This review aims at summarizing this body of knowledge, illuminating open questions and areas of development.

## Omega-3 FA: Natural substances, supplements or drugs?

Polyunsaturated FA present in nature belong to 2 main classes: the omega-6 (or n-6) class, mostly present in vegetable oils; and the omega-3 (or n-3) class, coming mostly, in modern Western diets, from fish. All these are "essential" FA, because they are necessary for an optimal health status and cannot be synthesized de novo. In addition, mammals cannot con-

vert omega-6 into omega-3 FA. In the chloroplasts of green leaves, algae and the phytoplankton, the conversion of linoleic acid (C18:2 n-6) into  $\alpha$ -linolenic acid (C18:3 n-3) can occur. Linoleic and  $\alpha$ -linolenic acids are the parent omega-6 and omega-3 FA, respectively. Their elongation and desaturation yield arachidonic acid (C20:4 n-6) and eicosapentaenoic acid (C20:5 n-3, EPA) in the omega-6 and the omega-3 classes, respectively. Eicosapentaenoic can be further elongated and desaturated to docosahexaenoic acid (C22:6 n-3, DHA), the most abundant omega-3 FA in the body. Omega-3 FA enter the food chain and become abundant in marine foods through the ingestion of marine phytoplankton by fish, thus yielding the most biologically active elongated products, EPA and DHA. Although most vegetable oils are abundant in linoleic acid, some of them (flaxseed, rapeseed [canola], linseed and perilla oils), and some nuts, contain abundant  $\alpha$ -linolenic acid, which our body can elongate and desaturate—to a certain (likely limited) extent—to EPA and DHA. At present, the most certain alimentary source of EPA and DHA is fish or meat from fish-eating animals (eg, seals for Eskimos).<sup>2-4</sup> These foods have been the main entry source of these FA into our alimentary chain for the 2 to 4 million years of the evolution of Homo Sapiens. The dramatic increase in the omega-6 to omega-3 FA ratio, which occurred after the introduction of modern agriculture and animal farming techniques, may have contributed to the rise in cardiovascular disease occurring after the industrial revolution. It has been argued that the nutritional recommendation of the National Heart Lung and Blood Institute to eat more polyunsaturated FA, which has resulted in introducing more omega-6 FA, may have caused detrimental health consequences by pushing towards an even more unbalanced omega-6 to omega-3 ratio.<sup>2</sup>

Cardiovascular health benefits of omega-3 FA may arise from their effects on atherogenesis, inflammation, thrombosis and target organ damage.<sup>2-5</sup> Recent analysis of a large American cohort of initially healthy women enrolled in the Nurses' Health Study<sup>6</sup> and from an Ital-

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ian case-control study<sup>7</sup> provide evidence of beneficial effects of these substances in nonfatal acute myocardial infarction, also; clearly a nonarrhythmic end point. Discussion of these issues is, however, out of the scope of the present review.

Aside from food, omega-3 FA may be obtained through supplements based on fish oil concentrates. Highly concentrated (up to 85%) preparations are now also available as prescription-only drugs, delivering relatively large amounts of these compounds in one or a few capsules per day. It is, however, unlikely that biological effects obtained in any clinical studies differ because of the use of different sources of these compounds. Being natural substances, omega-3 FA cannot be patented. Manufacturers have, however, patented the technical procedures that yield the concentrated compounds used in some studies. Preparations used may make some difference when issues of long-term compliance arise because large doses of these compounds are difficult to obtain from natural sources.

## Experimental evidence

In 1985, Murnaghan reported that, contrary to saturated FA, the addition of  $\alpha$ -linolenic acid to the perfusate of isolated rabbit hearts *in vitro* increased the threshold for arrhythmias.<sup>8</sup> McLennan et al were, however, the first to show that feeding rats for several months with fish oil omega-3 FA prevented fatal ventricular arrhythmias induced by ischemia in the isolated-perfused hearts of those rats.<sup>9</sup> These findings were repeatedly confirmed,<sup>10-13</sup> and also obtained in marmoset monkeys.<sup>14</sup> In rat feeding studies, McLennan et al showed that protection from ventricular fibrillation induced by the ligation of a coronary artery was around 70% when the intake of saturated or monounsaturated fats was replaced by vegetable oils rich in omega-6 FA, but virtually complete with fish oil.<sup>15</sup> These observations spurred further investigations, some in an animal model of sudden death in the dog. In this model, a myocardial infarction is produced surgically by ligating the left main coronary artery; an inflatable cuff is at the same time placed around the left circumflex artery. After a 1-month recovery from surgery, the dogs are trained to run on a treadmill. Around 60% of these animals are reproducibly susceptible to fatal ventricular fibrillation upon occlusion of the left circumflex artery during exercise. Dogs undergoing ventricular fibrillation are resuscitated with a direct-current electrical shock.<sup>16</sup>  $\beta$ -Blockers (possibly the only "classic" antiarrhythmic drugs demonstrated to be capable of preventing sudden death<sup>17</sup>) are protective in this model.<sup>18</sup> In susceptible animals, the intravenous infusion, just before exercise, of an emulsion of an omega-3 FA concentrate,<sup>19</sup> or of pure EPA, DHA or  $\alpha$ -linolenic acid delivered with serum albumin

as a carrier,<sup>20</sup> almost totally prevented ventricular fibrillation.

## Clinical evidence

While these studies were being conducted, the increasing awareness of the potential general health benefits of omega-3 FA was prompting intervention trials in cardiovascular disease. Most such trials concentrated on patients recovering from an acute myocardial infarction, where the risk of an ischemic event is higher. Three important clinical trials, the Diet and Reinfarction Trial (DART), the Lyon Heart Study, and the GISSI-Prevenzione Study, have progressively reinforced the belief of a preventive effect of omega-3 FA on sudden death. At the same time, retrospective analyses of previous trials, new small uncontrolled trials, and studies on surrogate end points of sudden death were conducted. Such studies (retrieved from a comprehensive Medline search), will be briefly reviewed here.

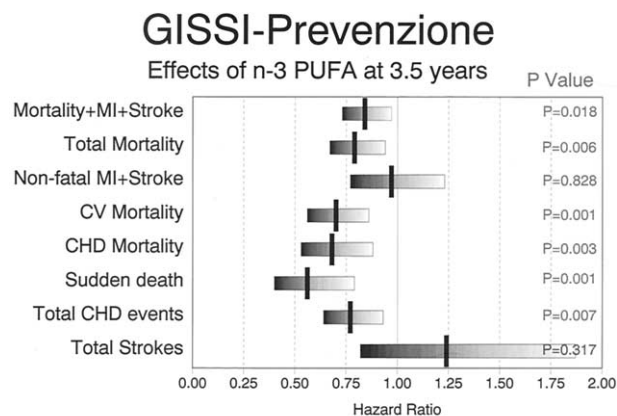
## Observational epidemiology and cohort case-control studies

Low rates of cardiovascular disease in populations with a high fish intake, such as Greenland Eskimos,<sup>21</sup> Alaskan natives,<sup>22</sup> and Japanese residing in fishing villages,<sup>23,24</sup> have originally suggested that fish consumption may protect against cardiovascular disease. Several,<sup>25-31</sup> but not all,<sup>32-36</sup> prospective cohort studies have found an inverse association between fish consumption and the risk of coronary heart disease (CHD). Inconsistencies have been ascribed to different methods of assessing diet and categorizing fish consumption: differences in study sites and times (involving associated dietary differences, eg, on the intake of cholesterol, saturated fats, antioxidants and fiber, which might have influenced the relation between fish consumption and CHD risk); and differences in follow-up duration, ranging from 4 years to several decades. Overall, most studies have shown an inverse association between fish consumption and CHD risk. However, in regard to whether the protective benefits are stronger for sudden (mostly arrhythmic) death<sup>37</sup> or nonsudden death, findings differ: Daviglus et al found an association especially with nonsudden death<sup>30</sup>; the Physician's Health Study found an association especially with sudden death<sup>31</sup>; and the Nurses' Health Study found an association with both, albeit more strongly with sudden death.<sup>6</sup>

At that time, a case-control study confirmed an inverse relationship of fish consumption and EPA levels in plasma with sudden cardiac death, suggesting a causal link.<sup>38</sup>

Most recently, in a 17-year follow-up of the Physicians' Health Study,<sup>31</sup> the fatty-acid composition of previously collected blood was analyzed in 94 men in

Figure 1



2-way analysis with interaction term

Effects of omega-3 FA on the primary (total mortality, myocardial infarction, and stroke) and secondary end points in the GISSI-Prevenzione study. (Used with permission from R. Marchioli on behalf of the GISSI-Prevenzione Investigators<sup>1</sup>).

whom sudden death occurred as the first manifestation of cardiovascular disease and in 184 age-matched and smoking-habit-matched controls. Baseline blood levels of long-chain omega-3 FA were inversely related to the risk of sudden death, both before and after adjustment for potential confounders.<sup>39</sup>

In aggregate, these studies suggest that fish consumption, through an increase in omega-3 FA, favorably affects cardiovascular risk, and that the benefit is at least partly due to the reduction of the risk of sudden death.

### Prospective studies

Four prospective studies have been performed so far.

The DART was a randomized, multifactorial, dietary-intervention study of >2000 Welsh men with a recent acute myocardial infarction. Three types of dietary interventions were tested over 2 years: (1) a reduction in fat intake; (2) an increase in the consumption of fiber; (3) an increase in fatty fish intake to at least 2 fish meals weekly. Subjects randomized to the fish-eating arm, but intolerant to fish, were allowed to take fish oil capsules. At 2 years, the men eating fish had significantly lower mortality (29%) compared with the other groups.<sup>40</sup> This effect was all ascribed to a reduction in CHD mortality. Mortality due to arrhythmias was not recorded, but because there was no reduction in new infarcts, the effect was attributed to a reduction in arrhythmic deaths.

The Lyon Heart Study was a prospective, randomized, single-blinded, secondary prevention study comparing the effect of a Mediterranean  $\alpha$ -linolenic-rich diet to the usual prudent postinfarct diet. The 302 subjects receiving the Mediterranean diet had a remarkable 70% reduction in all-cause mortality and morbidity, including the prevention of sudden death (8 in the control group, 0 in the experimental cohort). The active dietary component in this trial is difficult to sort out because of the complex dietary differences between the experimental groups, including an increased intake of grains, fruits and vegetables (rich in antioxidants), alcohol (mostly from red wine), and omega-3 FA (mostly  $\alpha$ -linolenic acid). An increase in omega-3 FA was found in plasma phospholipids in the group allocated to the Mediterranean diet, suggesting that these FA may be related to the observed benefit.<sup>41</sup>

It has been remarked that the divergence of the survival curves in the DART and the Lyon Study occurs in the first few weeks or months of the trial.<sup>42</sup> This contrasts with the  $\geq 2$  years taken for this separation to occur in most cholesterol-lowering trials.<sup>43-47</sup>

The Indian Experiment on Infarct Survival (Indian Study) randomized a very small number of patients ( $n = 122$ ) to 1.08 g/day of fish oil, or to mustard oil (rich in  $\alpha$ -linolenic acid, 2.9 g/day), or placebo. After 1 year, total cardiac events were reduced in the fish oil and mustard oil groups, but only the fish oil group had a significant ( $P < .05$ ) decrease in total cardiac and arrhythmic deaths compared to controls.<sup>48</sup>

The GISSI-Prevenzione Study randomized 11,324 patients who survived a recent myocardial infarction to supplements of omega-3 FA ( $n = 2836$ ), vitamin E ( $n = 2830$ ), both ( $n = 2830$ ), or none (control,  $n = 2828$ ) for 3.5 years. The primary combined efficacy end point was death, nonfatal myocardial infarction, and nonfatal stroke. Intention-to-treat analyses were done according to a factorial (2-way) design and by individual (4-way) treatments. The omega-3 fatty acid supplement, 1.0 g capsules of an 85% ethyl ester concentrate of fish oil FA, significantly reduced the primary end point by 10% (2-way analysis) and 15% (4-way analysis). Treatment lowered the relative risk of death 14% (2-way analysis) and 20% (4-way analysis), and cardiovascular death 17% or 30% by 2- and 4-way analysis, respectively. Though not a stated primary end point, there was a 45% reduction in sudden cardiac deaths (4-way analysis). There were no significant benefits for the vitamin E supplement (300 mg/day). The decrease in mortality by omega-3 FA derived from a 20% reduction in total deaths, 30% reduction in cardiovascular deaths, and a 45% reduction in sudden deaths<sup>1</sup> (Figure 1). In a recently published reanalysis of this study, survival curves were found to significantly diverge early after randomization; similar to what occurred in the Lyon Heart Study. Total mortality was

**Table I.** Characteristics of main prospective trials of omega-3 FA in the secondary prevention of myocardial infarction

Acronym/title	Comparisons	Enrollment criteria	No.	Follow-up	End points	Main results	Ref
DART/ Diet and Reinfarction Trial	1) Reduction in fat intake; 2) increase in fatty fish intake; 3) increase in cereal fibre intake; 4) control group: placebo	AMI, age <70 years, clinical stability	2033	2 years	Total mortality, IHD death, nonfatal MI	Eating fatty fish reduces total and cardiovascular mortality	40
Lyon/ Lyon Diet Heart Study	1) Mediterranean $\alpha$ -linolenic-rich diet; 2) control: usual prudent postinfarct-diet	AMI, age <70 years, clinical stability	605	27 months	Cardiac death, nonfatal MI, noncardiac death, all-cause death	Mediterranean $\alpha$ -linolenic-rich diets reduce 1) all-cause and cardiovascular mortality; 2) recurrence of MI and cardiac death	41, 42
Indian/ Indian Experiment of Infarct Survival	1) Fish oil; 2) mustard oil; 3) control group: placebo	AMI	406	1 year	Total cardiac death, total cardiac events, arrhythmic death	Fish oil reduces total cardiac and arrhythmic deaths	48
GISSI-Prevenzione/ Gruppo Italiano per lo Studio della Sopravvivenza nell' Infarto	1) Omega-3 fatty acids, 1 g/day; 2) vitamin E, 300 mg/day; 3) omega-3 fatty acids $\pm$ vitamin E; 4) control group: placebo	AMI	11324	3.5 years	Death, nonfatal MI, non-fatal stroke	Treatment with omega-3 FA supplement reduces 1) death plus nonfatal infarction and stroke; 2) mortality; 3) sudden death (not a primary end point)	1

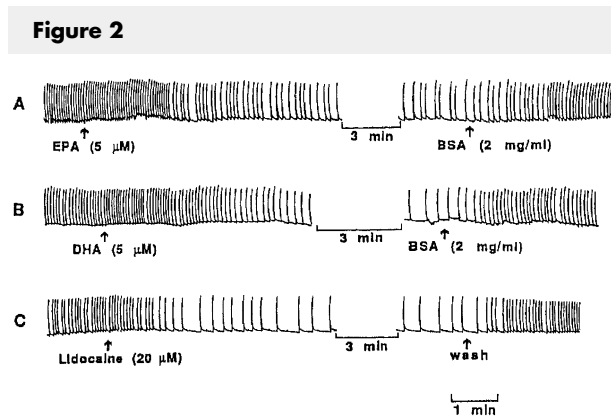
AMI, Acute myocardial infarction; IHD, ischemic heart disease; MI, myocardial infarction; Ref, reference.

found to be significantly lower after 3 months in the omega-3 FA group (relative risk [RR] 0.59, 95% CI 0.36-0.97,  $P = .037$ ). The reduction in risk of sudden death was relevant and statistically significant already at 4 months (RR 0.47, 95% CI 0.219-0.995,  $P = .048$ ).<sup>49</sup>

A comparison of the different features of these prospective trials is reported in Table I.

The GISSI-Prevenzione Study has been particularly important in supporting the idea of omega-3 FA as potential antiarrhythmic agents for several reasons:

- The study tested the clinical efficacy of 1 single dietary component, omega-3 FA, out of the many potentially active components in fish that were possibly involved in explaining the results of the DART and the Lyon Heart Study.
- The study was by far larger and had a longer follow-up than any previous such trial, and used a highly concentrated omega-3 fatty acid preparation.
- In the study, postmyocardial-infarction patients were treated according to best current practice, with aspirin,  $\beta$ -blockers, angiotensin-converting enzyme inhibitors, and cholesterol-lowering medications used to a very large extent.<sup>1</sup> The study therefore indicated a site of action of omega-3 FA different from that of other current therapies.
- The study led to reassessment of previous claims on the optimal dietary dose of omega-3 FA. After earlier studies on fish and the risk of CHD,<sup>25-31</sup> a claim had been made that maximal benefit is achieved by 1 meal per week of oily fish, with no further benefits from additional consumption.<sup>31,36</sup> At baseline, 73% of all 4 experimental groups in the GISSI-Prevenzione were ingesting  $\geq 1$  fish servings per week. At the 42-months study-termination point, this figure had increased to 87%. Despite this high basal level of fish ingestion, the further addition of omega-3 FA resulted in the significant beneficial effects reported.<sup>50</sup>
- Finally, the GISSI-Prevenzione Study was conducted in a population that largely adopted intensive preventive measures, including Mediterranean dietary habits,



Effects of omega-3 on the arrhythmic actions of calcium ions and the cardiac glycoside ouabain on cultured neonatal rat cardiomyocytes. Both these agents cause contracture and fibrillation of myocytes. However, when EPA is added before calcium or ouabain, there is a slowing of the beating rate and fibrillation is prevented (**A** and **B**). When both calcium and ouabain are added to the supernate, they induce a violent arrhythmia, which is terminated by the addition of EPA (**C**). When the free fatty acid is removed from myocytes by addition of delipidated bovine serum albumin (BSA), the violent arrhythmia promptly resumes. From: Kang JX and Leaf A.<sup>55</sup> Copyright 1994, National Academy of Sciences, USA.

with large proportions of foods allocated to fruits and vegetables, nonrefined carbohydrates and relatively low amounts of meat. Its findings therefore suggest additive cardiovascular benefit of the Mediterranean diet and omega-3 FA, and also that the high dietary intake in olive oil, rich in the monounsaturated fatty acid oleate, may favor (or synergize) the effects of omega-3 FA. This may occur because ingestion of high amounts of oleate would compete—in the diet or within cell membranes<sup>51,52</sup>—with omega-6 FA. Such combined action would help shift the omega-6/omega-3 ratio from the  $\geq 15:1$  that has been estimated for current American diet,<sup>53</sup> to the “ideal” 1:1. This is the ratio roughly estimated to have been present during the  $\geq 2$  million years when our forebears were adapting their genes to their environment (including their diet).<sup>2,53</sup>

## Clues to mechanisms

The idea that omega-3 FA may be effective antiarrhythmic agents, able to prevent ventricular arrhythmias, which cause the majority of sudden cardiac deaths, is now supported by a host of experimental evidence in vitro (more comprehensively reviewed by Leaf et al<sup>54</sup>).

## Experiments on cultured cardiomyocytes

When separated by enzymatic digestion and plated, cultured neonatal rat cardiac myocytes, by the second day of culture, grow in clumps, each consisting of a few to several hundred adherent interconnected cells that contract spontaneously, rhythmically and synchronously.<sup>55</sup> “Arrhythmias” can be produced in this model by a number of chemicals known to produce fatal ventricular fibrillation in humans, including elevated extracellular  $[Ca^{2+}]$ , toxic concentrations of ouabain (mimicking digitalis toxicity), the  $\beta$ -adrenergic agonist isoproterenol, lysophosphatidylcholine, thromboxane  $A_2$ , and the calcium ionophore A23187—these last 2 compounds act by directly increasing intracellular  $[Ca^{2+}]$ . The addition of polyunsaturated FA to the culture medium in low micromolar concentrations (achievable by feeding or supplementation) prevents these cardiomyocyte “arrhythmias.” Whereas omega-3 FA are antiarrhythmic in all instances,<sup>55</sup> omega-6 FA are so only in the presence of cyclooxygenase blockade, because prostaglandins and thromboxane  $A_2$ , produced from arachidonic acid through cyclooxygenase, are mostly proarrhythmogenic. This agrees with the results of McLennan et al, which showed that vegetable oils, rich in omega-6 FA, have only partial antiarrhythmic properties.<sup>15</sup> The free fatty acid removal (by adding delipidated albumin) abrogates the antiarrhythmic effect. An example of these experiments is shown in Figure 2. Structural requirements for these in vitro antiarrhythmic effects are a long acyl chain with  $\geq 2$  C=C unsaturated bonds and a free carboxyl group at one end. The presence of the free fatty acid in the system is required.<sup>56</sup>

## Effects of polyunsaturated FA on ion currents

Prevention of in vitro induced arrhythmias by omega-3 FA can be explained by complex modulation of sarcolemmal ionic channels, whose interplay determines cardiac action potential (Figure 3).

Omega-3 FA concentration-dependently inhibit the  $Na^+$  current ( $I_{Na}$ ), producing a large voltage-dependent shift (10–20 mV) in the potential for one half steady-state inactivation ( $V_{1/2}$ ) to a more hyperpolarized value.<sup>57</sup> The effect is not use-dependent, as expected for lipophilic modulators, and occurs with an  $IC_{50}$  of 5  $\mu M$  in neonatal rat cardiomyocytes,<sup>58</sup> and an  $IC_{50}$  of 0.5  $\mu M$  in an embryonic cell line expressing the human myocardial hH1 $\alpha$   $Na^+$  channel  $\alpha$ -subunit.<sup>59</sup> Omega-3 FA stabilize the inactivated state of the channel, accelerating the transition from the resting to the inactivated state: the estimated affinity of EPA for the inactivated state is  $>250$ -fold higher than for the resting state.<sup>54</sup> At the cellular level, the threshold voltage for  $Na^+$  channel opening is set to a more positive value, and the effective refractory period of the cardiac cycle is prolonged. In addition, a slight hyperpolarization of

the diastolic resting potential is observed, tentatively attributed to a decrease in background  $\text{Na}^+$  current.<sup>54</sup>

The L-type  $\text{Ca}^{2+}$  channel is also affected. Electrophysiological effects on L-type  $\text{Ca}^{2+}$  current ( $I_{\text{Ca,L}}$ ) are similar to those observed for  $I_{\text{Na}}$ , consisting of a concentration-dependent current inhibition, with a negative shift of the steady-state inactivation curve. This effect has been observed for EPA and DHA at concentrations around 1  $\mu\text{M}$ , but not with saturated or mono-unsaturated FA.<sup>60,61</sup> Other actions of omega-3 FA on  $\text{Ca}^{2+}$  homeostasis have not been convincingly demonstrated.<sup>12,54,61,62</sup>

Evidence of  $\text{K}^+$  channel modulation has also been provided. Whole-cell voltage clamp experiments (reviewed by Leaf et al<sup>54</sup>) showed inhibition of the transient outward ( $I_{\text{to}}$ ) and delayed rectifier ( $I_{\text{K}}$ ) currents, but not of the inward rectifying current ( $I_{\text{K1}}$ ). However, decreased  $\text{K}^+$  efflux is expected to increase action potential duration, whereas either no change or even a slight decrease of action potential duration has been recorded in cardiomyocytes exposed to omega-3 FA.<sup>63</sup>

Finally, some ligand-gated channels, namely the cAMP-dependent  $\text{Cl}^-$  channel and the acetylcholine-dependent  $\text{K}^+$  channel, may be inhibited by omega-3 FA.<sup>54</sup>

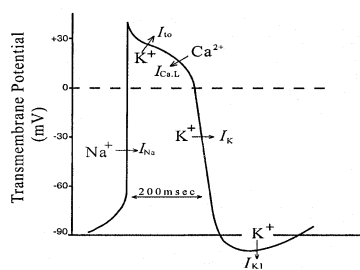
The molecular mechanisms responsible for all these reported actions have not been fully elucidated. The effect on the  $\text{Na}^+$  channel was abolished by a single amino acid substitution in the  $\alpha 1$  subunit (N406K),<sup>64</sup> suggesting a specific interaction with the channel protein. However, because omega-3 FA can modulate a multitude of different voltage-gated or ligand-gated channels, it seems likely that nonspecific mechanisms are also operating. Alterations of phospholipid package (sometimes referred to as changes in membrane “fluidity”) have been detected,<sup>65</sup> but they occur only at high, nonphysiological, concentrations. An alternative possibility is an interference with lipid microdomains located in close contiguity to the channel proteins, where a sort of molecular “strain” has been speculated to exist due to a mismatch between the length of the hydrophobic transmembrane portion of the protein and the thickness of the lipid bilayer.<sup>66,67</sup> By binding to membrane phospholipids at this level, omega-3 FA might decrease such strain, leading to conformational changes in the channels. Consistent with this view, detergents that are chemically unrelated to omega-3 FA have produced similar effects on  $\text{Na}^+$  and  $\text{Ca}^{2+}$  currents.<sup>68</sup>

Arrhythmias triggered by ischemia or ischemia-reperfusion are caused by the interplay of several basic mechanisms, namely increased automaticity inside or outside the sinoatrial node, triggered activity (sustained by early or delayed afterdepolarizations), and reentry.<sup>69</sup> The reported electrophysiological effects of

**Figure 3**

Effects of n-3 PUFAs on Ion Currents through Membrane Channels in Cardiomyocytes

Ion Current	Function	Effect on /	$I_{\text{C}_{50}}$
1. Voltage-gated $\text{Na}^+$ current, $I_{\text{Na}}$	Initiates action potential	↓	( $\mu\text{M}$ )
a. Neonatal rat myocyte			4.8
b. Human $\alpha$ -subunit			0.51
2. L-type $\text{Ca}^{2+}$ Current, $I_{\text{Ca,L}}$	Action potential plateau	↓	
a. Neonatal rat	$\text{Ca}^{2+}$ induced $\text{Ca}^{2+}$ release	↓	0.8
b. Adult rat	Cytosolic $\text{Ca}^{2+}$ fluctuations	↓	2.1
3. $\text{K}^+$ Currents			
a. Transient outward currents, $I_{\text{to}}$	Repolarizes myocyte	↓	7.5
b. Slow delayed rectifier current, $I_{\text{K}}$	Repolarizes myocyte	↓	20.0
c. Inward rectifier current, $I_{\text{K1}}$	Stabilizes resting potential	none	



Effects of omega-3 FA on ion currents through membrane channels in cardiomyocytes. Basic electrophysiological mechanisms underlying the various phases of the cardiac action potential are shown in the figure, depicting a typical action potential in a cardiomyocyte (see Ravens et al<sup>95</sup> for details). The upper portion details experimentally documented effects of omega-3 FA on ion currents. (Modified with permission from Leaf et al.<sup>96</sup>)

omega-3 FA may conceivably translate into an antiarrhythmic action.  $\text{Na}^+$  channel inhibition is expected to decrease automaticity, because of membrane hyperpolarization associated with increased threshold voltage for  $\text{Na}^+$  channel opening. In addition, a prolongation of the effective refractory period might interfere with reentry circuits. L-type  $\text{Ca}^{2+}$  channel modulation is also relevant, because ischemia causes increased free  $\text{Ca}^{2+}$  concentration in the cytosol (cytosolic  $\text{Ca}^{2+}$  “overload”) and oscillations in cytosolic  $\text{Ca}^{2+}$  concentration, major determinants of delayed afterdepolarizations.<sup>69</sup> The inhibition of  $I_{\text{Ca,L}}$  might reduce  $\text{Ca}^{2+}$  overload, chiefly by reducing the sarcoplasmic reticulum  $\text{Ca}^{2+}$  load, thus preventing delayed afterdepolarizations. Indeed, omega-3 FA reduce time-averaged cytosolic  $\text{Ca}^{2+}$  concentration and fluctuations in cytosolic  $\text{Ca}^{2+}$  in cardiomyocytes exposed to lysophosphatidylcholine,  $\beta$ -adrenergic agonists, or ouabain.<sup>55,56</sup>

Interestingly, omega-3 FA appear to counteract the proarrhythmic effects of other lipid metabolites.<sup>68</sup> Amphiphilic metabolites, particularly lysophosphatidylcholine, which increases during ischemia, enhance

inward currents (especially the  $\text{Na}^+$  current) and inhibit ionic transporters (especially the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger and  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase), causing membrane depolarization, reduction of upstroke velocity, and delayed afterdepolarizations. On the other hand, arachidonic acid and related FA determine action potential shortening (chiefly by activating outward  $\text{K}^+$  currents) and favor the development of  $\text{Ca}^{2+}$  overload, probably by stimulating the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger.

### Clues to the occurrence of antiarrhythmic effects of omega-3 FA on surrogate end points of sudden death and open issues

The occurrence of an antiarrhythmic effect of omega-3 FA in humans specifically linked to the prevention of sudden death is still undemonstrated. Supporting evidence for this comes from the population-based, case-control study by Siscovick et al, which included 334 case patients with primary cardiac arrest and 493 controls. In this study, a moderate dietary intake of omega-3 FA (about 1 fatty fish meal/week) was associated with a significant 50% reduction in the risk of primary cardiac arrest.<sup>38</sup>

There have been attempts to study the occurrence of antiarrhythmic effects of omega-3 FA on surrogate end points of sudden death in humans. One obvious end point is the occurrence of non-life-threatening arrhythmias, such as ventricular premature contractions (VPCs) recorded at continuous ECG (Holter) monitoring. This is based on the fact that the occurrence of frequent and complex VPCs and ventricular tachycardias has been recognized as a hallmark of life-threatening ventricular fibrillation, both in the absence<sup>70</sup> and in the presence of thrombolysis.<sup>71</sup> One small trial with omega-3 FA has shown a trend towards VPC reduction,<sup>72</sup> and another has shown a significant reduction.<sup>73</sup> In this latter study, 34 patients with good left ventricular function and frequent but not high-grade VPCs, without other life-threatening arrhythmias, were given a total of 2.4 g/d omega-3 FA for 16 weeks; 34 similar patients were given a sunflower seed oil (rich in the omega-6 fatty acid linoleate) as control. Twenty-four hour Holter monitoring, performed before and after treatment, showed that VPCs decreased 48% in the fish-oil group and 25% in controls. The percentage of patients having a clinically relevant >70% reduction in VPCs was higher in the fish-oil group than in the control group (44% vs 15%,  $P < .05$ ).<sup>73</sup> The validity of VPC reduction in predicting favorable outcomes for sudden death or cardiac arrest is, however, disputable. The Cardiac Arrhythmia Suppression Trial (CAST) tested the hypothesis that agents that reduce the frequency of VPCs or other arrhythmias at Holter moni-

toring also reduce mortality. Contrary to expectations, 2 such drugs, the class I agents flecainide and moricizine, actually increased mortality in patients with CHD.<sup>74</sup>

However, some other evidence for the occurrence of antiarrhythmic effects of omega-3 FA in humans comes from reports on the increase in heart rate variability by omega-3 FA. A low heart rate variability is a powerful predictor of total mortality, sudden death and arrhythmic events in patients after an acute myocardial infarction,<sup>75-79</sup> and also in healthy subjects.<sup>80</sup> A recent consensus report on this marker stated that, because of its stability and the absence of a placebo effect, 24-hour heart rate variability may be ideal for the assessment of intervention therapies.<sup>78</sup>  $\beta$ -Blockers, repeatedly reported to reduce mortality after myocardial infarction,<sup>81</sup> clearly increase this index.<sup>82-86</sup> In a highly consistent series of studies, Christensen et al have shown an effect of omega-3 FA on heart rate variability in humans. First, in 55 postmyocardial infarction patients with left ventricular dysfunction, these authors documented a direct relationship between habits of fish consumption (and corresponding omega-3 FA levels in platelet membranes) and heart rate variability, expressed as the standard deviation of the R-R interval on 24-hour Holter monitoring.<sup>87</sup> Secondly, a randomized treatment of these high-risk patients with omega-3 FA (4.3 g of EPA and DHA) significantly increased heart rate variability.<sup>88</sup> Thirdly, in patients with chronic renal failure on dialysis, who have an even lower heart rate variability than postmyocardial infarction patients with left ventricular dysfunction,<sup>89</sup> Christensen et al have shown that patients with the highest heart rate variability also had the highest omega-3 FA levels in cell membranes.<sup>90,90a</sup> Omega-3 FA also appeared to increase heart rate variability in healthy subjects.<sup>91</sup>

How omega-3 FA increase heart rate variability is not obvious. Their effects on the inward sodium current and the L-type calcium channel, if also occurring on cardiac pacemaker cells of the sinus node, could decrease heart rate, which is by itself a predictor of mortality in various categories of cardiac patients<sup>75-77,87,92,93</sup> and not clearly independent of heart rate variability. Studies on ion currents and experiments in the dog model of sudden death suggest that a shift in the autonomic balance toward a reduced sympathetic and increased vagal activity occurs with omega-3 FA. Similar to  $\beta$ -blockers, a bradycardic effect may also carry along an effect of increased heart rate variability. It has been suggested that a rate-independent effect of a drug on heart rate variability can be established by demonstrating an effect on heart rate variability normalized for the heart rate (eg, expressing the standard deviation of the R-R intervals as a percent of the mean R-R interval, ie, its coefficient of variation).<sup>86</sup> Such correc-

tions have not yet been clearly reported in the setting of omega-3 FA, and further research is warranted.

### Future directions

Several types of evidence are converging to indicate potential for omega-3 FA in the prevention of fatal ventricular arrhythmias. This hypothesis, backed-up by observational epidemiology and case-control studies, a host of in vitro and animal-experiment models, and prospective clinical studies (which are, however, not yet specifically testing the antiarrhythmic hypothesis), is now being evaluated in at least 3 ongoing clinical trials in patients with intracardiac devices (implantable defibrillators). These patients are at the highest risk for developing fatal ventricular arrhythmias. In these cases the implantable defibrillator would likely protect against otherwise fatal episodes of ventricular tachycardia or fibrillation, and also record the number and characteristics of such episodes. These patients therefore appear ideal to test the antiarrhythmic hypothesis in a formal way. It is also interesting to note that the best study available to date that suggests omega-3 FA prevent sudden death, the GISSI-Prevenzione study, was deliberately carried out in a population of patients with a recent myocardial infarction without evident left-ventricular dysfunction. At the time the GISSI-Prevenzione study was being planned, the main hypotheses for the use of omega-3 FA were their antiatherogenic and antithrombotic properties. Because of this, the inclusion of patients less likely to die because of a progression of atherosclerosis and coronary thrombosis would have diluted an effect of treatment. Retrospectively, however, such inclusion criteria in the GISSI-Prevenzione study (pointing towards subjects at low risk of sudden death) has probably been a bias against the demonstration of a larger difference between treatment and control groups. The GISSI Group (Tavazzi L et al, personal communication) is therefore starting another study specifically designed to test the hypothesis of a mortality reduction by omega-3 FA in subjects with heart failure, who are at higher risk of arrhythmic death.

### Clinical implications

Sudden cardiac death, largely due to ventricular fibrillation, accounts for some 250,000 to 300,000 deaths annually in the United States.<sup>37</sup> Most patients with a fatal acute coronary episode die before reaching the hospital, in large part because of a malignant ventricular arrhythmia.<sup>94</sup> Prevention of prehospital death from an acute ischemic episode is therefore an important target for future interventions in cardiology, and should be addressed by preventive strategies along

with the goal of reducing the total CHD burden. The challenge of identifying candidates for sudden death among subjects not previously identified as patients with CHD is currently largely unmet. Having been part of the human diet for most of the 2 to 4 million years of our evolution,<sup>2</sup> omega-3 FA are exceptionally safe. There is therefore a potentially large public health benefit from the use of omega-3 FA in primary cardiovascular prevention. Therefore, although these compounds should be in use already, as of now, in all patients after a first myocardial infarction, randomized prospective studies in primary prevention are warranted.

### Addendum

Since the final submission of the current review, recent findings have further reinforced the hypothesis of cardiovascular protective effects of omega-3 FA, in part acting through a decrease in sudden, likely arrhythmic, death.

In a prospective, nested case-control analysis in apparently healthy men followed-up for up to 17 years in the Physician's Health Study, the fatty acid composition of previously collected blood was analyzed in 94 men in whom sudden death occurred as the first manifestation of cardiovascular disease and in 184 controls matched for age and smoking status. Baseline blood levels of long-chain omega-3 FA were inversely related to the risk of sudden death even after adjustment for potential confounders.<sup>97</sup>

Lemaitre et al<sup>98</sup> investigated the associations of plasma phospholipids concentration of DHA, EPA and  $\alpha$ -linolenic acid with the risk of incident fatal ischemic heart disease and incident nonfatal myocardial infarction in older adults in a nested case-control study within the Cardiovascular Health Study. Fifty-four cases of incident fatal ischemic heart disease death were matched with 125 incident nonfatal acute myocardial infarction and 179 randomly selected, matched controls. Higher concentrations of combined DHA and EPA were associated with a lower risk of fatal ischemic heart disease, a higher concentration of  $\alpha$ -linolenic acid was associated with a trend towards lower risk, while none of these fatty acids was associated with nonfatal myocardial infarction. In a subsequent analysis from the same study, the inverse association of fish consumption with ischemic heart disease (especially arrhythmic) death was limited to the consumption of tuna or other broiled or baked fish, but not fried fish or fish sandwiches, a finding possibly related to the preparation method increasing the omega-6:omega-3 ratio, levels of trans-fatty acids, and other lipid oxidation products.<sup>99</sup>

A higher consumption of fish and long-chain omega-3 FA was associated with a lower CHD incidence

and total mortality among women with diabetes.<sup>100</sup> In this study, a higher fish consumption was associated with a significantly lower risk of both fatal CHD (multivariate relative risk for  $\geq 5$  fish meals per week 0.41, 95% CI 0.18–0.94) and, notably, nonfatal myocardial infarction (multivariate relative risk for  $\geq 5$  fish meals per week 0.28, 95% CI 0.11–0.71). This reinforces the contention that cardiovascular health effects of fish eating and omega-3 FA are not totally accounted for by the reduction in arrhythmic death.<sup>101</sup>

## References

1. The GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamins E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 1999; 354:447–55.
2. Leaf A, Weber PC. A new era for science in nutrition. *Am J Clin Nutr* 1987;45:1048–53.
3. De Caterina R, Endres S, Kristensen S, et al. n-3 Fatty acids and vascular disease. New York: Springer; 1993.
4. Kristensen S, Endres S, De Caterina R, et al. n-3 Fatty acids: prevention and treatment in vascular disease. New York: Springer; 1995.
5. De Caterina R, Zampolli A. n-3 fatty acids: antiatherosclerotic effects. *Lipids* 2002;36(Suppl):S69–78.
6. Hu F, Bronner L, Willet WC, et al. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA* 2002;287:1815–21.
7. Tavani A, Pelucchi C, Negri E, et al. n-3 Polyunsaturated fatty acids, fish, and nonfatal acute myocardial infarction. *Circulation* 2001;104:2269–72.
8. Murnaghan MF. Effects of fatty acids on the ventricular arrhythmia threshold in the isolated heart of the rabbit. *Br J Pharmacol* 1985;73:909–15.
9. McLennan PL, Abeywardena MY, Charnock JS. Dietary fish oil prevents ventricular fibrillation following coronary artery occlusion and reperfusion. *Am Heart J* 1988;116:709–17.
10. Hock C, Beck L, Bodine L, et al. Influence of dietary n-3 fatty acids on myocardial ischemia and reperfusion. *Am J Physiol* 1990; 259:H1518–61.
11. Yang B, Saldeen G, Bryant L, et al. Dietary fish oil supplementation attenuates myocardial dysfunction and injury in isolated rat hearts. *J Nutr* 1993;123:2067–74.
12. Kinoshita I, Itoh K, M N-N, et al. Antiarrhythmic effects of eicosapentaenoic acid during myocardial infarction: enhanced cardiac microsomal ( $\text{Ca}^{2+}$ - $\text{Mg}^{2+}$ )-ATPase activity. *Japan Circ J* 1994;58: 903–12.
13. Anderson KE, Du X-J, Sinclair AJ, et al. Dietary fish oil prevents reperfusion  $\text{ins}(1,4,5)\text{-P}_3$  release in rat heart: possible antiarrhythmic mechanism. *Am J Physiol* 1996;271:H1843–490.
14. McLennan PL, Bridle TM, Abeywardena MY, et al. Dietary lipid modulation of ventricular fibrillation threshold in the marmoset monkey. *Am Heart J* 1992;123:1555–61.
15. McLennan PL. Relative effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on cardiac arrhythmias in rats. *Am J Clin Nutr* 1993;57:207–12.
16. Billman GE, Schwartz PJ, Stone HL. Baroreceptor reflex control of heart rate: a predictor of sudden cardiac death. *Circulation* 1989;66:874–80.
17. Teo K, Yusuf S, Furberg C. Effects of prophylactic antiarrhythmic drug therapy in acute myocardial infarction: an overview of results from randomized controlled trials. *JAMA* 1993;270:1589–95.
18. Schwartz P. Do animal models have clinical value? *Am J Cardiol* 1998;81:14–20D.
19. Billman GE, Kang JX, Leaf A. Prevention of ischemia-induced cardiac sudden death by n-3 polyunsaturated fatty acids. *Lipids* 1997;32:1161–8.
20. Billman GE, Kang JX, Leaf A. Prevention of ischemia-induced cardiac sudden death by pure n-3 polyunsaturated fatty acids. *Circulation* 1999;99:2452–7.
21. Kromann N, Green A. Epidemiological studies in the Upernavik District, Greenland. *Acta Med Scand* 1980;208:401–6.
22. Middaugh J. Cardiovascular deaths among Alaskan natives, 1980–1986. *Am J Public Health* 1990;80:282–5.
23. Hirai A, Hamazaki T, Terano T, et al. Eicosapentaenoic acid and platelet function in Japanese. *Lancet* 1980;2:1132–3.
24. Kagawa Y, Nishizawa M, Suzuki M, et al. Eicosapolyenoic acids of serum lipids of Japanese islanders with low incidence of cardiovascular disease. *J Nutr Sci Vitaminol* 1982;28:441–53.
25. Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N Engl J Med* 1985;312:1205–9.
26. Kromhout D, Feskens E, Bowles C. The protective effect of a small amount of fish on coronary heart mortality in an elderly population. *Int J Epidemiol* 1995;24:340–5.
27. Shekelle R, Missel L, Paul O, et al. Fish consumption and mortality from coronary heart disease. *N Engl J Med* 1985;313:820–4.
28. Norell S, Ahlbom A, Feychting M, et al. Fish consumption and mortality from coronary heart disease. *BMJ* 1986;293:426.
29. Shekelle R, Stamler J. Fish and coronary heart disease: the epidemiologic evidence. *Nutr Metab Cardiovasc Dis* 1993;3:46–51.
30. Daviglus M, Stamler J, Orenca A, et al. Fish consumption and the 30-year risk of fatal myocardial infarction. *N Engl J Med* 1997;336:1046–53.
31. Albert CM, Hennekens CH, O'Donnell CJ, et al. Fish consumption and sudden cardiac death. *JAMA* 1998;279:23–8.
32. Vollset S, Heuch I, Bjelke E. Fish consumption and mortality from coronary heart disease. *N Engl J Med* 1985;313:820–1.
33. Curb J, Reed D. Fish consumption and mortality from coronary heart disease. *N Engl J Med* 1985;313:821–2.
34. Fraser G, Sabate J, Beeson W, et al. A possible protective effect of nut consumption on risk of coronary heart disease: the Adventist Health Study. *Arch Intern Med* 1992;152:1416–24.
35. Morris M, Manson JE, Rosner B, et al. Fish consumption and cardiovascular disease in the Physicians' Health Study: a prospective study. *Am J Epidemiol* 1995;142:166–75.
36. Ascherio A, Rimm EB, Stampfer MJ, et al. Dietary intake of marine n-3 fatty acids, fish intake, and the risk of coronary heart disease among men. *N Engl J Med* 1995;332:977–82.
37. American Heart Association. Heart and stroke facts: 1997 statistical supplement. American Heart Association: Dallas; 1997.
38. Siscovick DS, Raghunathan TE, King I, et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA* 1995;274: 1363–7.
39. Albert C, Campos H, Stampfer M, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med* 2002;346:1113–8.

40. Burr M, Gilbert JF, Holliday RM, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet* 1989;334:757-61.
41. de Lorgeril M, Renaud S, Mamelle N, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* 1994;343:1454-9.
42. de Lorgeril M, Renaud S, Martin J-L, et al. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* 1999;99:779-85.
43. The Lipid Research Clinics Study Group. The lipid research clinics coronary primary prevention trial results: I. Reduction in incidence of coronary heart disease. *J Am Med Assoc* 1984;251:351-64.
44. Sacks F, Pfeffer M, Moye A, et al. The effects of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. *N Engl J Med* 1996;335:1001-9.
45. Shepherd J, for the West of Scotland Coronary Prevention Study Group. The West of Scotland coronary prevention study: a trial of cholesterol reduction in Scottish men. *Am J Cardiol* 1995;76:113-7C.
46. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: The Scandinavian simvastatin survival study (4S). *Lancet* 1994;344:1383-9.
47. Scandinavian Simvastatin Survival Study Group. Baseline serum cholesterol and treatment effect in the Scandinavian simvastatin survival study (4S). *Lancet* 1995;345:1274-5.
48. Singh RB, Niaz MA, Sharma JP, et al. Randomized, double-blind, placebo-controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: the Indian experiment of infarct survival. *Cardiovasc Drugs Ther* 1997;3:485-91.
49. Marchioli R, Barzi F, Bomba E, et al. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation* 2002;105:1897-903.
50. Leaf A, De Caterina R, von Schacky C. The GISSI-Prevenzione trial: letter to the Editor. *ISSFAL Newsletter* 2000;332:977-82.
51. Massaro M, De Caterina R. Vasculoprotective effects of oleic acid: epidemiological background and direct vascular antiatherogenic properties. *Nutr Metab Cardiovasc Dis* 2002;12:42-51.
52. Leaf A. On the reanalysis of the GISSI-Prevenzione. *Circulation* 2002;105:1874-5.
53. Simopoulos A. Evolutionary aspects of diet and essential fatty acids. In: Hamazaki T, Okuyama H, editors. *Fatty acids and lipids: new findings*. Basel, Switzerland: Karger; 2001. p. 18-27.
54. Leaf A, Kang J, Xiao Y-F, et al. The antiarrhythmic and anticonvulsant effects of dietary n-3 fatty acids. *J Membrane Biol* 1999;172:1-11.
55. Kang JX, Leaf A. Effects of long-chain polyunsaturated fatty acids on the contraction of neonatal rat cardiac myocytes. *Proc Natl Acad Sci USA* 1994;91:9886-90.
56. Kang JX, Leaf A. Prevention and termination of arrhythmias induced by lysophosphatidyl choline and acylcarnitine in neonatal rat cardiac myocytes by free omega-3 polyunsaturated fatty acids. *Eur J Pharmacol* 1996;297:97-106.
57. Bendahhou S, Cummins T, Agnew W. Mechanism of modulation of the voltage-gated skeletal and cardiac muscle sodium channels by fatty acids. *Am J Physiol* 1997;272:C592-600.
58. Xiao Y-F, Kang JX, Morgan JP, et al. Blocking effects polyunsaturated fatty acids on Na<sup>+</sup> channels of neonatal rat ventricular myocytes. *Proc Natl Acad Sci USA* 1995;92:11000-4.
59. Xiao Y-F, Wright SN, Wang GK, et al. N-3 fatty acids suppress voltage-gated Na<sup>+</sup> currents in HEK293t cells transfected with the  $\alpha$ -subunit of the human cardiac Na<sup>+</sup> channel. *Proc Natl Acad Sci USA* 1998;95:2680-5.
60. Hallaq H, Smith TW, Leaf A. Modulation of dihydropyridine-sensitive calcium channels in heart cells by fish oil fatty acids. *Proc Natl Acad Sci USA* 1992;89:1760-4.
61. Xiao Y-F, Gomez AM, Morgan JP, et al. Suppression of voltage-gated L-type Ca<sup>2+</sup> currents by polyunsaturated fatty acids in adult and neonatal rat cardiac myocytes. *Proc Natl Acad Sci USA* 1997;94:4182-7.
62. Swanson JE, Lokesh BR, Kinsella JE. Ca<sup>2+</sup>-Mg<sup>2+</sup>-ATPase of mouse cardiac sarcoplasmic reticulum is affected by membrane n-6 and n-3 polyunsaturated fatty acid content. *J Nutr* 1989;119:364-72.
63. Kang J, Xiao Y, Leaf A. Free long-chain polyunsaturated fatty acids reduce membrane electrical excitability in neonatal rat cardiac myocytes. *Proc Natl Acad Sci USA* 1995;92:3997-4001.
64. Xiao Y, Ke Q, Wang S, et al. Single point mutation affects fatty acid block of human sodium  $\alpha$ -subunits Na<sup>+</sup> channels. *Proc Natl Acad Sci USA* 2001;98:3606-11.
65. Klausner R, Kleinfeld A, Hoover R, et al. Lipid domains in membranes. *J Biol Chem* 1980;255:1286-95.
66. Lundbaek J, Birn J, Girshman P, et al. Membrane stiffness and channel function. *Biochemistry* 1996;35:3825-30.
67. Lundbaek J, Andersen O. Spring constants for channel-induced lipid bilayer deformations: estimates using gramicidin channels. *Biophys J* 1999;76:889-95.
68. Leaf A, Xiao Y. The modulation of ionic currents in excitable tissues by n-3 polyunsaturated fatty acids. *J Membr Biol* 2001;184:263-71.
69. Carmeliet E. Cardiac ionic currents and acute ischemia: from channels to arrhythmias. *Physiol Rev* 1999;79:917-1017.
70. Bigger J, Fleiss J, Kleiger R, et al. The relationship among ventricular arrhythmias, left ventricular dysfunction, and mortality in the 2 years after myocardial infarction. *Circulation* 1984;69:250-8.
71. Maggioni A, Zuanetti G, Franzosi M, et al. Prevalence and prognostic significance of ventricular arrhythmias after acute myocardial infarction in the fibrinolytic era: GISSI-2 results. *Circulation* 1993;87:312-22.
72. Christensen JH, Gustenhoff P, Eilersen E. n-3 Fatty acids and ventricular extra systoles in patients with ventricular tachyarrhythmias. *Nutr Res* 1995;15:1-8.
73. Sellmayer A, Witzgall H, Lorenz R, et al. Effects of dietary fish oil on ventricular premature complexes. *Am J Cardiol* 1996;76:974-7.
74. The Cardiac Arrhythmia Suppression Trial (CAST) Investigators. Preliminary report: effect of encainide and flecainide on mortality in a randomised trial of arrhythmias suppression after myocardial infarction. *N Engl J Med* 1989;321:406-12.
75. Kleiger RE, Miller JP, Bigger JT, et al. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol* 1987;59:256-62.
76. Farrell TG, Bashir Y, Cripps T, et al. Risk stratification for arrhythmic events in postinfarction patients based on heart rate variability, ambulatory electrocardiographic variables and the signal-averaged electrocardiogram. *J Am Coll Cardiol* 1991;18:687-97.

77. Hartikainen JK, Malik M, Staunton A, et al. Distinction between arrhythmic and nonarrhythmic death after acute myocardial infarction based on heart rate variability, signal-averaged electrocardiogram, ventricular arrhythmias and left ventricular ejection fraction. *J Am Coll Cardiol* 1996;28:296-304.
78. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation* 1996;93:1043-65.
79. La Rovere MT, Bigger JJT, Marcus FI, et al. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. *Lancet* 1998;351:478-84.
80. Mølgaard H, Sørensen KE, Bjerregaard P. Attenuated 24-h heart rate variability in apparently healthy subjects subsequently suffering sudden cardiac death. *Clin Auton Res* 1991;1:233-7.
81. Waldo AL, Camm AJ, deRuyter H, et al. Effect of d-sotalol on mortality in patients with left ventricular dysfunction after recent or remote myocardial infarction. *Lancet* 1996;348:7-12.
82. Burger A, Kamalesh M. Effect of beta-adrenergic blocker therapy on the circadian rhythm of heart rate variability in patients with chronic stable angina pectoris. *Am J Cardiol* 1999;83:596-8.
83. Iacoviello M, Massari F, De Laura D, et al. Influence of hydrophilic and lipophilic beta-blockers on heart rate, ventricular repolarization and their interrelationships in normal subjects. *It Heart J* 2000;1:331-5.
84. Lin J, Chan H, Du C, et al. Long-term beta-blocker therapy improves autonomic nervous regulation in advanced congestive heart failure: a longitudinal heart rate variability study. *Am Heart J* 1999;137:658-65.
85. Weber F, Schneider H, von Arnim T, et al. Heart rate variability and ischemia in patients with coronary heart disease and stable angina pectoris: influence of drug therapy and prognostic value: the total ischemic burden bisoprolol (TIBB) study. *Eur Heart J* 1999;20:38-50.
86. Vaile J, Fletcher J, Al-Ani M, et al. Use of opposing reflex stimuli and heart rate variability to examine the effects of lipophilic and hydrophilic beta-blockers on human cardiac vagal control. *Clin Sci (Colch)* 1999;97:585-93.
87. Christensen JH, Gustenhoff P, Korup E, et al. Fish consumption, n-3 fatty acids in cell membranes, and heart rate variability in survivors of myocardial infarction with left ventricular dysfunction. *Am J Cardiol* 1997;79:1671-3.
88. Christensen JH, Gustenhoff P, Korup E, et al. Effect of fish oil on heart rate variability in survivors of myocardial infarction: a double blind randomized controlled trial. *BMJ* 1996;312:677-8.
89. Gruppo Patologie Cardiovascolari ed Emodialisi. Multicentre, cross-sectional study of ventricular arrhythmias in chronically haemodialysed patients. *Lancet* 1988;2:305-9.
90. Christensen JH, Aarøe J, Knudsen N, et al. Heart rate variability and n-3 fatty acids in patients with chronic renal failure: a pilot study. *Clin Nephrol* 1998;49:102-6.
- 90a. Christensen JH, Skou HA, Fog L, et al. Marine n-3 fatty acids, wine intake, and heart rate variability in patients referred for coronary angiography. *Circulation* 2001;103:651-7.
91. Christensen J, Christensen M, Dyerberg J, et al. Heart rate variability and fatty acids content of blood cell membrane: a dose-response study with n-3 fatty acids. *Am J Clin Nutr* 1999;70:331-7.
92. Barron HV, Viskin S. Autonomic markers and prediction of cardiac death after myocardial infarction. *Lancet* 1998;351:461-2.
93. Zuanetti G, Mantini L, Hernandez-Bernal F, et al. Relevance of heart rate as a prognostic factor in patients with acute myocardial infarction: insights from the GISSI-2 study. *Eur Heart J* 1998;19:F19-26.
94. Myerburg RJ, Kessler KM, Castellanos A. Sudden cardiac death: epidemiology, transient risk and intervention assessment. *Ann Intern Med* 1993;119:1187-97.
95. Ravens U, Wettwer E, Ohler A, et al. Electrophysiology of ion channels of the heart. *Fundam Clin Pharmacol* 1996;10:321-8.
96. Leaf A. How n-3 fatty acids prevent cardiac arrhythmias. *CVD/Lipid Dialog* 1998;8:1-7.
97. Albert C, Campos H, Stampfer M, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med* 2002;346:1113-8.
98. Lemaitre RN, King IB, Mozaffarian D, et al. n-3 polyunsaturated fatty acids, fatal ischemic heart disease, and nonfatal myocardial infarction in older adults: the cardiovascular health study. *Am J Clin Nutr* 2003;77:319-25.
99. Mozaffarian D, Lemaitre RN, Kuller LH, et al. Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the cardiovascular health study. *Circulation* 2003;107:1372-7.
100. Hu FB, Cho E, Rexrode KM, et al. Fish and long-chain omega-3 fatty acid intake and risk of coronary heart disease and total mortality in diabetic women. *Circulation* 2003;107:1852-7.
101. Hu FB, Willet WC. Optimal diets for prevention of coronary heart disease. *JAMA* 2002;288:2569-78.