

## Prevention of Cardiac Arrhythmia by Dietary (n-3) Polyunsaturated Fatty Acids and Their Mechanism of Action<sup>1,2</sup>

Sudheera S. D. Nair, James W. Leitch\*, John Falconer\*\* and Manohar L. Garg<sup>3</sup>

*Discipline of Nutrition and Dietetics, University of Newcastle, Callaghan, NSW 2308, Australia, \*Department of Cardiology and \*\*Discipline of Reproductive Medicine, John Hunter Hospital, NSW 2308, Australia*

**ABSTRACT** The role of marine fish oil (n-3) polyunsaturated fatty acids in the prevention of fatal ventricular arrhythmia has been established in experimental animals. Prevention of arrhythmias arising at the onset of ischemia and reperfusion is important because if untreated, they result in sudden cardiac death. Animals supplemented with fish oils in their diet developed little or no ventricular fibrillation after ischemia was induced. Similar effects have also been observed in cultured neonatal cardiomyocytes. Several mechanisms have been proposed and studied to explain the antiarrhythmic effects of fish oil polyunsaturated fatty acids, but to date, no definite mechanism has been validated. The sequence of action of these mechanisms and whether more than one mechanism is involved is also not clear. Some of the mechanisms suggested to explain the antiarrhythmic action of fish oils include the incorporation and modification of cell membrane structure by (n-3) polyunsaturated fatty acids, their direct effect on calcium channels and cardiomyocytes and their role in eicosanoid metabolism. Other mechanisms that are currently being investigated include the role of (n-3) polyunsaturated fatty acids in cell signalling mediated through phosphoinositides and their effect on various enzymes and receptors. This article reviews these mechanisms and the antiarrhythmic studies using (n-3) polyunsaturated fatty acids. *J. Nutr.* 127: 383–393, 1997.

**KEY WORDS:** • (n-3) fatty acids • cardiac arrhythmia • electrophysiology • cardiovascular disease • humans • dogs • rats • marmoset monkeys

Cardiovascular disease (CVD)<sup>4</sup> describes all diseases of the heart and blood vessels including heart disease, stroke and peripheral vascular disease, and is the leading cause of death in western nations. The increased consumption of fats, particularly saturated fats, in the diets of industrialized and developing nations has been linked to the increase in deaths due to coronary heart disease. Epidemiological studies, human intervention trials and animal experiments have shown that dietary fatty acids can modify the risk for cardiovascular disease.

Though there has been a fall in mortality from coronary heart disease in recent years, sudden cardiac deaths associated with fatal arrhythmia remains the cause of most deaths in industrialized societies (Charnock 1991). Mortality statistics from the USA and UK indicate that up to 80% of sudden deaths are due to ventricular fibrillation. Studies such as the cardiac arrhythmia suppression trial (CAST) failed to show

any significant decrease in mortality due to coronary artery disease when antiarrhythmic drugs were administered.

Heart rhythms are a result of waves of electrical excitation which spread through the conducting tissues in heart muscle. Individual heart cells known as cardiac myocytes are electrically coupled to each other by membrane structures called gap junctions, which are small pores through which electrical currents can flow from cell to cell. When a region of the heart becomes ischemic, the electrical properties change, leading to arrhythmias. The most common fatal arrhythmia is known as ventricular fibrillation (VF), in which electrical impulses from damaged cardiac muscle cause the normal synchronicity of heart contractions to break down. It is believed that at least half of the deaths due to coronary artery disease in the United States are caused by disturbances in the electrical stability of the heart, terminating in VF (Billman and Leaf 1994). Cardiac arrhythmia occurs during the early and potentially reversible phase of ischemia (Sargent and Riemersma 1990) and after reperfusion. In most cases arrhythmia occurs without previous symptoms and progresses to sudden cardiac death.

**Dietary lipids and (n-3) fatty acids.** Lipids are important dietary constituents and in the body serve as efficient sources of energy when stored in adipose tissue. They are also required by the body for cell structure and membrane function and as a source of precursors for eicosanoid synthesis. Constituents of lipids like cholesterol and phospholipids regulate membrane-associated functions such as activities of membrane bound enzymes, receptors, ion channels, etc. (Clandinin et al. 1991). Lipids are composed of fatty acids of different chain lengths

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<sup>3</sup> To whom correspondence and reprint requests should be addressed.

<sup>4</sup> Abbreviations used: AA, arachidonic acid; CSR, cardiac sarcoplasmic reticulum; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EET, epoxyeicosatetraenoic acid; EPA, eicosapentaenoic acid; FFA, free fatty acid; IP<sub>3</sub>, inositol triphosphate; LA, linoleic acid;  $\alpha$ -LNA, alpha linolenic acid; LT, leukotriene; NEFA, non-esterified fatty acids; PG, prostaglandin; PUFA, polyunsaturated fatty acids; RAC, receptor agonist complex; TX, thromboxane; VF, ventricular fibrillation; VFT, ventricular fibrillation threshold.

and degrees of saturation as well as different configurations. The degree of unsaturation among lipids is of great interest to lipid researchers because of its effect on health. The most significant characteristic of dietary lipids is the content of different types of fatty acids.

Fatty acids are classified into three families: saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids (PUFA). The common saturated fatty acids in our diet are myristic, palmitic and stearic acids derived largely from animal fats, dairy products and manufactured foods. The monounsaturated fatty acid content of our diet is accounted for by oleic acid, the predominant component of canola oil, olive oil and sunola. All mammals can synthesize saturated fatty acids and monounsaturated fatty acids *de novo* from simple precursors such as glucose or amino acids using fundamental pathways. Linoleic acid [18:2(n-6); LA] and  $\alpha$ -linolenic acid [18:3(n-3);  $\alpha$ -LNA], the precursors of the (n-6) and (n-3) family of fatty acids, respectively, are essential fatty acids and have to be supplied by the diet. The main PUFA in the western diet is linoleic acid, found mostly in vegetable oils such as safflower oil, sunflower oil, cottonseed oil, corn oil, soybean oil, etc.

Significant amounts of  $\alpha$ -LNA are found in green vegetables and in vegetable oils like linseed oil, canola oil and soybean oil. Longer chain PUFA, like arachidonic acid [20:4(n-6); AA], are synthesized in human tissues via chain elongation and desaturation of LA. Arachidonic acid may also originate as such from muscle meat membranes, eggs, organ meats and human milk. Eicosapentaenoic acid [20:5(n-3); EPA] and docosahexaenoic acid [22:6(n-3); DHA] are synthesized via desaturation and chain elongation of  $\alpha$ -LNA and are also found in high concentrations in fish and fish oils. The metabolism of fatty acids of the (n-3) family (EPA and DHA) and of the (n-6) family (AA) are of particular interest because of the actions of their metabolites (eicosanoids) *in vivo*. Double bond positions of (n-6) and (n-3) fatty acids are not interconvertible. LA and  $\alpha$ -LNA compete for desaturation and chain elongation, therefore, a proper balance is essential to optimize AA and DHA in the membranes.

**Epidemiological studies linking (n-3) fatty acids and CVD.** Epidemiological studies have demonstrated a link between fish oil consumption and reduced risk of CVD (Bang and Dyerberg 1976). Several intervention trials (Dolocek and Grandits 1991, Keys 1980, Kromhout et al. 1985, Shekelle et al. 1985) have been carried out to confirm this and also to explore the mechanisms by which fish oil may prevent CVD. The well known effects of fish oil in reducing hypertriglyceridemia (Phillipson et al. 1985) and thrombosis (Goodnight 1986, Von Schacky and Weber 1985) may partly explain this preventive effect. Fish oil contains highly polyunsaturated fatty acids that are vulnerable to oxidation, and thus can be expected to increase the risk of CVD. Therefore, a definite mechanism by which marine oil prevents CVD is not clear at present. Recent animal studies have demonstrated that fish oil may prevent cardiac arrhythmias, which could provide an explanation for the reduced CVD mortality in fish-eating populations like the Greenland Inuits and the Japanese.

**Intervention trials linking (n-3) fatty acids and cardiac arrhythmias.** In the first controlled trial in which dietary advice on fish consumption was given, the Diet and Reinfarction Trial (DART), 2.5 g of EPA weekly (corresponding to about 300 g fatty fish per week) was found to be successful in reducing mortality by 29% in men during the first two years after myocardial infarct, measured by susceptibility to VF (Burr et al. 1989). In a recent study in Seattle, WA, the red blood cell membrane fatty acid levels, a biomarker of dietary intake of

(n-3) fatty acids, were analyzed in 334 patients with primary cardiac arrest. The study found that compared to no intake of dietary EPA and DHA, a monthly intake of 5.5 g of (n-3) fatty acids (equivalent to one fatty fish meal per week) was associated with a 50% reduction in the risk of primary cardiac arrest (Siscovick et al. 1995). In the Lyon Diet Heart Study, de Lorgeril and colleagues (1994) compared the effect of a Mediterranean  $\alpha$ -LNA rich diet in postinfarct patients. They found that patients assigned to the Mediterranean diet had a significant reduction in the rate of recurrence of cardiac events and overall mortality. The antiarrhythmic effect of (n-3) PUFA in humans has been confirmed by another recent study (Sellmayer et al. 1995). A placebo-controlled, double blind study was conducted to assess the effect of dietary (n-3) PUFA on the frequency of ventricular premature complexes in patients with good ventricular function who experienced frequent but not life threatening ventricular arrhythmias. The patients were randomly assigned to receive either fish oil (a total of 2.4 g (n-3) PUFA/d containing 1.5 g EPA and 0.9 g DHA) for 16 wk or sunflower seed oil as placebo. They found that ventricular premature complexes decreased by 48% in the fish oil group and by 25% in the placebo group.

**Models for arrhythmogenic studies.** In recent years, the work of McLennan and colleagues (McLennan et al. 1990, McLennan et al. 1985, 1988, 1992, 1993, 1995, 1996, Pepe and McLennan 1996) and Leaf and co-workers (Billman et al. 1994, Hallaq et al. 1990, Kang and Leaf 1994, 1995, 1996, Leaf 1995) have made major inroads into the study of (n-3) PUFA and cardiac arrhythmias. They have established a definite link between (n-3)PUFA from fish oil and prevention of arrhythmias in experimental animals. These studies are summarized in **Table 1** and **Table 2**.

The antiarrhythmic effects of fish oil in experimental animals have focussed primarily on two models of arrhythmia. They are 1) ischemia induced (arrhythmias occurring after onset of ischemia) and 2) reperfusion arrhythmias.

Other areas in which the effects of (n-3) PUFA are currently being studied include heart transplants (Grimminger et al. 1996) and restenosis after coronary angioplasty (Leaf et al. 1994). These studies have attempted to elucidate the prophylactic mechanism of fish oil (n-3) PUFA by studying effects on cardiac function, mechanical performance of the heart and ventricular fibrillation threshold. Several mechanisms have been suggested to explain the antiarrhythmic action of (n-3) PUFA, but to date no definite mechanism has been fully validated.

## ANIMAL EXPERIMENTS

**Induced ischemia model.** Experimental animals including rats, marmoset monkeys and dogs have been used to demonstrate the effects of different fatty acids on cardiac arrhythmia. In these experiments, ischemia was induced by coronary artery occlusion.

Abeywardena et al. (1993) examined the effect of long term dietary supplementation of different dietary fatty acids on arrhythmia in rats and marmoset monkeys. The study demonstrated that cardiac eicosanoids were significantly reduced by fish oil feeding, and this subsequently reduced ventricular fibrillation. The association between consumption of different diets and the vulnerability of the myocardium to develop arrhythmias was also studied in adult marmoset monkeys (McLennan et al. 1992). An increase in threshold current required to induce VF was observed after inducing ischemia in animals fed sunflower seed oil diet [(n-6) PUFA] or tuna fish oil diet [(n-3) PUFA] compared to animals fed a saturated fat diet.

TABLE 1

*Effect of (n-3) polyunsaturated fatty acid supplementation on arrhythmia in experimental animals<sup>1</sup>*

Reference	Animal model	Diet treatment	Amount of supplementation	Duration of supplementation	Electrical property examined	Effect
Pepe and McLennan 1996	Rats	Purified fish oil	12 g/100 g diet	16 wk	VF VFT	↓↓ ↑↑
McLennan et al. 1996	Rats	DHA ethyl ester EPA ethyl ester DHA ethyl ester	1.1% energy 1.1% energy 0.3% energy + EPA ethyl ester 0.4% energy	5 wk 5 wk 5 wk	VF VF VF	↓↓ No effect ↓↓
McLennan and Dallimore 1995	Rats	Canola oil	12 g/100 g diet	12 wk	VF	↓
McLennan 1993	Rats	Fish oil	12 g/100 g diet	12 wk	VPB VF	↓↓ ↓↓
Yang et al. 1993a	Rats	Fish oil preparation [40% (n-3) PUFA]	12 g/100 g diet	5 d	VF	↓↓
Yang et al. 1993b	Rats	Fish oil preparation [40% (n-3) PUFA]	12 g/100 g diet	4–5 wk	Cardiac dynamics Cardiac contraction	↓ ↓
Paulson et al. 1992	Rats	Menhaden oil	5 g/100 g diet	15 wk	Coronary perfusion pressure Cardiac contractile performance	↓ Improved recovery
Charnock et al. 1991	Rats	SF/FO	2.1 g/100 g diet	44 wk	VPB VT VF	↓↓ ↓↓ No effect
Sargent and Riemersma 1990	Rats	Purified fish oil	7% energy	8 wk	VF	↓↓
McLennan et al. 1990	Rats	Tuna fish oil	12 g/100 g diet	9 mo	VT VF	↓↓ ↓↓
Hock et al. 1990	Rats	Menhaden oil	5% energy	4 wk	VF VT	↓↓ ↓↓
McLennan et al. 1988	Rats	Tuna fish oil	12 g/100 g diet	12 wk	VEB VFT VF VT	↓↓ ↑↑ No effect ↓↓
Hock et al. 1987	Rats	Menhaden oil	5% energy	4 wk	VEB	No effect
Charnock et al. 1992	Marmoset monkeys	Fish oil	6 g/100 g diet	24 mo	VFT	↑↑
McLennan et al. 1992	Marmoset monkeys	Tuna fish oil	18.8% energy		VF VFT	No effect ↑↑
McLennan et al. 1993	Marmoset monkeys	Purified fish oil	3 g/100 g diet	16 wk	VF VFT	↓↓ ↑↑
Billman et al. 1994	Mongrel dogs	Fish oil fatty acid emulsion	70% (n-3) PUFA	i.v. infusion 50–60 minutes prior	VF	↓↓
Kinoshita et al. 1994	Mongrel dogs	EPA ester	100 mg/kg · body wt	8 wk	VF	No effect
Culp et al. 1980	Mongrel dogs	Menhaden oil	25% energy	36–45 d	VEB & infarction	↓↓
Hartog et al. 1987	Pigs	Mackerel oil	4.5 g/100 g diet	16 wk	VPB	↓
Hartog et al. 1986	Pigs	Mackerel oil	9.1 g/100 g diet	8 wk	VPB	No effect

<sup>1</sup> Abbreviations used: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; FO, fish oil; SF, saturated fat; VF, ventricular fibrillation; VT, ventricular tachycardia; VFT, ventricular fibrillation threshold; VEB, ventricular ectopic beats; VPB, ventricular premature beats. ↓↓, substantial decrease; ↓, moderate decrease; ↑↑, substantial increase; ↑, moderate increase; VFT ↑↑ and VF ↓↓ = reduced arrhythmia vulnerability.

In a recent study by Pepe and McLennan (1996), hearts isolated from rats fed fish oil for 16 wk were set up as a working heart model. They found that dietary fish oil prevented and reduced the severity of arrhythmias in ischemia as well as increased the threshold for VF.

Charnock et al. (1992b) studied the effect of (n-3) PUFA on cardiac performance in marmoset monkeys and found the dietary treatment to be beneficial. Both the mechanical performance and the electric stability of animal hearts improved on the (n-3) PUFA diet. Turner et al. (1990) confirmed that the antiarrhythmic effect of dietary (n-3) PUFA is independent of atherosclerosis-induced cardiac vulnerability and is due to their effect on myocardial membrane alone. These studies support the need to further study the mechanism of action of these fatty acids and their influence on the cardiac environment.

**Ischemia-reperfusion model.** Reperfusion arrhythmias arise when total or partial blood flow is restored to a previously ischemic region of the heart (Nettleton 1995). Feeding rats fish oil (menhaden oil) for 4 wk was found to exert a beneficial effect on myocardial ischemia-reperfusion injury (Hock et al. 1990). The rats had a significant reduction in ischemic damage and incidence of fatal arrhythmias. The authors suggested this effect may be due to related changes in the fatty acid composition of myocardium, neutrophil and platelet phospholipid. Long term dietary modulation of rat myocardial membrane fatty acids was studied by McLennan et al. (1988). They found that tuna fish oil effectively reduced the vulnerability of the myocardium to both ischemic and reperfusion arrhythmias. In a similar study Yang et al. (1993a, b) observed that dietary fish oil supplementation was cardioprotective against ischemia and reperfusion. Re-

TABLE 2

*Effect of (n-3) polyunsaturated fatty acid supplementation on arrhythmia in vitro<sup>1</sup>*

Reference	Animal model	Diet treatment	Amount of supplementation	Duration of supplementation	Electrical property examined	Effect
Kang and Leaf	Cultured rat cardiomyocytes	Fish oil	3 $\mu\text{mol/L}$ EPA		LPC induced arrhythmia	No effect
Xiao et al. 1995	Cultured rat cardiomyocytes	Fish oil	10–15 $\mu\text{mol/L}$ EPA 5 $\mu\text{mol/L}$		Blocking effects on Na channel	$\downarrow\downarrow$ Moderate
Kang and Leaf 1995	Cultured rat cardiomyocytes	Fish oil	10 $\mu\text{mol/L}$ 5–10 $\mu\text{mol/L}$ EPA/DHA	2–3 min	Isoproterenol induced VF	$\downarrow\downarrow$ $\downarrow\downarrow$
Kang et al. 1995	Cultured rat cardiomyocytes	Fish oil	10 $\mu\text{mol/L}$ EPA	1–2 min	Depolarizing current	$\uparrow$
Kang and Leaf 1994	Cultured rat cardiomyocytes	Fish oil	5–10 $\mu\text{mol/L}$ EPA/DHA	3 min perfusion	Myocyte contraction rate	50–80% $\downarrow\downarrow$
Hallaq et al. 1992	Cultured rat cardiomyocytes	Fish oil	5–10 $\mu\text{mol/L}$ EPA/DHA		Ouabain induced arrhythmia (contraction rate)	$\downarrow\downarrow$
Hallaq et al. 1990	Cultured rat cardiomyocytes	Fish oil	5 $\mu\text{mol/L}$ EPA	3–5 d incubation in medium	Myocyte contraction rate	$\downarrow\downarrow$

<sup>1</sup> Abbreviations used: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LPC, lysophosphatidylcholine; VF, ventricular fibrillation;  $\downarrow\downarrow$ , substantial decrease;  $\downarrow$ , moderate decrease;  $\uparrow\uparrow$ , substantial increase;  $\uparrow$ , moderate increase.

perfusion damage has also been attributed to calcium overload (Naylor and Panagiotopoulos 1988), free radicals and calcium (Hearse and Tosaki 1988), thromboxane (Coker et al. 1982, Coker and Parrat 1985), disturbance of potassium and magnesium ion balance and effects on enzymes (Manning and Hearse 1984) and on the action of thrombin and inositol triphosphates (Jacobsen et al. 1996). But it should be mentioned here that the effects of dietary supplementation on most of these factors have not been examined.

**Effect of fish oils on ventricular fibrillation.** The antiarrhythmic properties of (n-3) PUFA have been studied in vivo by measuring the ventricular fibrillation threshold (VFT) of animals whose diets were supplemented with fish oils. VFT is the amount of current required to induce VF during myocardial ischemia. The antiarrhythmic actions of (n-3) PUFA have also been studied in vitro using cultured neonatal cardiac myocytes.

The work of Hallaq et al. (1990) using rat cardiac myocytes cultured in a medium supplemented with (n-3)PUFA showed that fish oil prevented VF, and this was attributed to the prevention of increase in cytosolic calcium by (n-3) PUFA. This prophylactic effect of fish oils on VF was studied using ouabain, a potent arrhythmogenic agent, in isolated neonatal cardiac myocytes. Ouabain is a cardiac glycoside and is toxic because it produces excessively high levels of calcium inside the cells. The toxic effects of ouabain were prevented in myocytes incubated with EPA.

Using mongrel dogs Billman et al. (1994) demonstrated that long chain (n-3) PUFA concentrated from fish oil prevented ischemia-induced VF. In that study fish oil fatty acids were infused intravenously before inducing ischemia, and the effect on VF was recorded. In seven of eight animals, the infusion of fish oil emulsion completely prevented acute occurrence of VF. Since the infusion with fish oil emulsion was carried out only 1 h prior to inducing ischemia, the authors suggested that the effects were not mediated via membrane incorporation of (n-3) PUFA but rather via a direct action of (n-3) PUFA on the electrophysiological properties of myocytes.

The effect of adrenergic stimulation on the development of ischemia-induced malignant arrhythmia was studied in isolated

multicellular cardiac myocyte preparations using fish oil (n-3) PUFA (Kang and Leaf 1995). In that study unesterified, long-chain PUFA, particularly (n-3) PUFA, but not monounsaturated or saturated fatty acids, were found to effectively prevent and terminate  $\beta$ -adrenergic agonist-induced tachyarrhythmias in myocyte preparations. McLennan et al. (1985) found that the number of ventricular ectopic beats and duration of tachycardia or fibrillation was increased in rats fed perirenal sheep fat compared to rats fed sunflower seed oil. In the same rat model, when rats were fed tuna fish oil, VF was prevented during occlusion and reperfusion of the coronary artery, and the severity of arrhythmia was significantly reduced (McLennan et al. 1988).

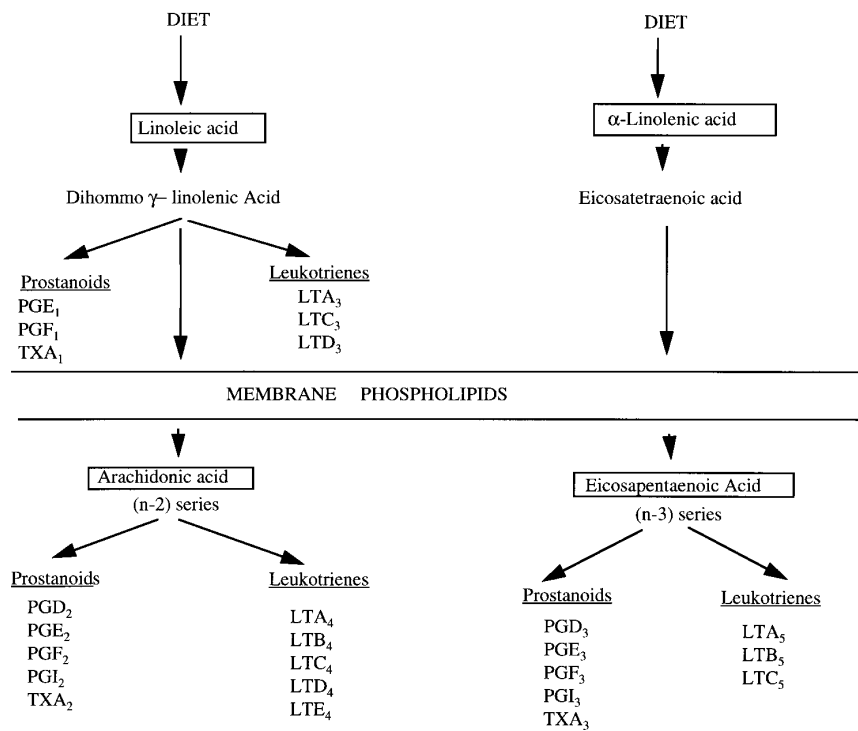
## MECHANISMS

The mechanisms suggested by different authors in their studies on arrhythmias can be broadly classified as follows:

- Modification of the eicosanoid system by dietary fatty acids.
- Modification of the fatty acid composition of membrane phospholipids.
- Direct effect of non-esterified fatty acids (NEFA) on the myocardium.
- Effect of (n-3) PUFA on the inositol lipid cycle and cell signalling.
- Effect of (n-3) PUFA on  $\text{Ca}^{2+}$  channels.
- Effect of (n-3) PUFA on enzymes and receptors.

It is generally agreed by most authors that the prevention of arrhythmias by fish oil is a result of the interaction of two or more of these mechanisms, but the sequence of action has not been elucidated. The effects of fish oil are most clearly and simply explained by their effects on eicosanoid metabolism, but it is also obvious that other factors interact at one point or other. It has been aptly said that (n-3) PUFA modify so many different factors, it is impossible to attribute all their beneficial effects to one single action (Leaf 1994).

**Effect of (n-3)PUFA on eicosanoid metabolism.** LA of the (n-6) family and the precursor of AA, is by far the dominant precursor fatty acid for eicosanoid formation provided by the



**FIGURE 1** Biosynthesis of eicosanoids from dietary fatty acids. LT, leukotriene; PG, prostaglandin; TX, thromboxane.

Western diet. In both animals and humans LA undergoes a series of elongation and desaturation to yield AA. The role of AA in arrhythmia is of particular interest. Most investigations of the link between fish oils and heart disease have demonstrated the competition between AA and (n-3) PUFA to become substrates in the production of eicosanoids. The biosynthesis of eicosanoids from fatty acids is presented in **Figure 1**. When fish oils are included in the diet, the (n-3) PUFA, EPA and DHA, compete with AA in several ways: 1) They inhibit  $\Delta$ -6 desaturase activity to inhibit AA biosynthesis (Garg et al. 1988). 2) They compete with AA for the sn-2 position in membrane phospholipid thereby reducing plasma and cellular levels of AA (Siess et al. 1988). 3) EPA competes with AA as the substrate for the cyclooxygenase enzyme inhibiting the production of thromboxane A<sub>2</sub> (TXA<sub>2</sub>) by platelets (Fischer and Weber 1983). 4) In endothelial cells, prostaglandin I<sub>3</sub> (PGI<sub>3</sub>) is synthesized from EPA which adds on to prostaglandin I<sub>2</sub> (PGI<sub>2</sub>) (Fischer and Weber 1984).

The net result of these actions is vasodilation with less platelet aggregation—an antithrombotic effect. By altering the availability of AA in the membrane, a change in the production of eicosanoids and eicosanoid dependent cellular functions may be produced. A reduced ratio of AA/EPA shifts the spectrum of eicosanoid production toward an increase in thromboxane A<sub>3</sub> (TXA<sub>3</sub>) and PGI<sub>3</sub> at the expense of TXA<sub>2</sub> and PGI<sub>2</sub>, respectively. This shift was found to reduce the risk of VF and sudden cardiac death (SCD) (Coker et al. 1982). The risk of ventricular arrhythmia induced by ischemia was found to be directly proportional to the balance between TXA<sub>2</sub> and PGI<sub>2</sub>. Coker and Parrat (1985) found that TXA<sub>2</sub> was released as an early response to occlusion while PGI<sub>2</sub> was released after the onset of ischemia. Drugs that blocked the TXA<sub>2</sub> receptor reduced incidence of ischemia-induced arrhythmia and reperfusion arrhythmia. When local PGI<sub>2</sub> activity was raised, the frequency and severity of both types of arrhythmia was reduced. Parrat et al. (1987) postulated that

an increase in PGI<sub>2</sub>/TXA<sub>2</sub> balance may be cardioprotective. They also suggest that PGI<sub>2</sub> may be an endogenous anti-arrhythmic agent, which may explain the preventive mechanism of (n-3) PUFA.

The effect of (n-3) PUFA on eicosanoids was also examined in cultured rat ventricular myocytes under normoxic and hypoxic conditions (Oudot et al. 1995). The work showed that (n-3)-rich cardiomyocytes displayed better electromechanical recovery during hypoxia and reoxygenation than (n-6)-rich cardiomyocytes. The authors suggested that dietary (n-3) PUFA contribute to the protection of the heart by modulating eicosanoid synthesis at both the vascular and cardiomyocyte levels.

Another antiarrhythmic mechanism suggested by Abeywardena et al. (1991) describes the existence of different substrate (fatty acid) pools as a result of different dietary lipid supplementation and an inhibitory action of (n-3) PUFA on thromboxane synthetase. The same authors (Abeywardena et al. 1993) examined the effects of various fatty acids on myocardial eicosanoids and on myocardial phospholipids. Fish oil-fed animals had the lowest proportion of LA and AA with a rise in (n-3) fatty acids in the cardiac membrane. These studies have highlighted the importance of the balance of eicosanoids in determining the arrhythmic outcome in ischemia as well as after reperfusion. A recent study by Pepe and McLennan (1996) of isolated perfused hearts from rats fed fish oil concurs with the above studies. Fish oil feeding prevented severe arrhythmias and increased the VF threshold. The authors speculated that the most likely mechanism in this study was the altered fatty acid composition of myocardial membranes or intracellular nonesterified fatty acid pools.

A study of the fatty acid composition of phospholipids of heart sarcolemma after rats were fed one of four different types of oil found that dietary fish oil, unlike the other oils, resulted in a complete change of the sarcolemmal fatty acid pattern (Al Makdessi et al. 1994). The balance of AA that was main-

tained by the other three oils, namely coconut oil, corn oil and linseed oil, was changed by the presence of the fish oils such that AA was displaced by EPA and DHA. Charnock et al. (1992a) demonstrated in marmoset monkeys that the proportions of AA, EPA and DHA in myocardial phospholipids can be altered by dietary fatty acids, which in turn leads to a shift in the balance between levels of PGI<sub>2</sub> and TXA<sub>2</sub>. Fish oil (n-3) PUFA promotes a balance that is beneficial to the prevention of cardiac arrhythmia. These beneficial changes in the marmoset heart were also accompanied by changes in the fatty acid composition of cardiac membrane phospholipids, which links this mechanism with the second mechanism in this review, namely the effect of (n-3) PUFA on membrane phospholipids.

Another mechanism that has not been explored and which may be investigated in future studies is the role of hydroxylated AA metabolites. AA differs from other fatty acids in that it is a substrate for cyclooxygenase, lipoxygenase and cytochrome P450 pathway enzymes that synthesize bioactive eicosanoids. These metabolites are important modulators of the cell signalling processes that play a major role in heart disease and arrhythmias. Epoxyeicosatetraenoic acid (EET) and hydroxyeicosatetraenoic acid are AA metabolites that are formed by the lipoxygenase and cytochrome P450 pathways. They have proinflammatory action and have been found to be a type of autocrine mediator that acts by altering membrane structure and function (Spector et al. 1994). EET has been found to produce rapid calcium flux in porcine smooth muscle cells into which they are taken up and incorporated into phospholipids. The EET are then inactivated by their conversion into dihydroxyeicosatrienoic acid, which terminates the effect of calcium influx and causes vascular relaxation (Spector et al. 1996). The effect of fish oil supplementation on the different AA pathways and metabolites and their possible role in the antiarrhythmic mechanism of (n-3) PUFA is a new area that needs to be explored.

**Effect of (n-3) PUFA on membrane phospholipids.** It is believed that changes in the lipid composition of biological membranes lead to changes in their function. This is true for the cardiac membrane (sarcolemma) also (Charnock 1994). Cardiac sarcolemma plays a major role in regulating the movement of ions entering and leaving the cell. Receptors involved in cellular signalling, transporters and enzymes are embedded in the membrane lipid bilayer, and any changes to the fatty acid composition of this membrane could affect their functions. The influence on cardiac membrane function by dietary lipids has been demonstrated in both man and experimental animals (Spector and Yorek, 1985).

It has been suggested that diet-induced changes in the fatty acid composition of cardiac muscle cell membranes are associated with development of arrhythmia (Charnock et al. 1985). Hallaq et al. (1992) observed that alterations in the fatty acid composition of the culture medium in which cardiac myocytes were grown also altered myocyte function. Changes in cardiac phospholipids and the NEFA composition brought about by manipulation of dietary fatty acids with (n-3) PUFA has been repeatedly demonstrated as antiarrhythmic (Charnock et al. 1992b, McLennan et al. 1990, McLennan 1993).

The mechanism postulated here is that changes in the lipid composition of cardiac cell membranes induced by dietary modification can influence the availability of Ca<sup>2+</sup> for excitation-contraction coupling, which in turn could lead to the development of the arrhythmic state. A diet supplemented with fish oil changes the phospholipid composition of the cardiac membrane by reducing the level of (n-6) PUFA and increasing the (n-3) PUFA levels. Membrane phospholipids

control the transfer of ions across the membrane, and fatty acid composition of membrane phospholipids may influence the properties of specific ion channels like the calcium and sodium channels (Hallaq et al. 1990).

The activation of phospholipase enzyme is another mechanism that has been proposed to explain degradation of cardiac membranes after long periods of ischemia. Grynberg et al. (1992) found that the activity of phospholipase in the cardiac cell could be influenced by phospholipid fatty acid composition. In their study using cardiomyocytes cultured separately in EPA- or DHA-supplemented media, they found that phospholipase activity was lower in the EPA medium under hypoxic conditions, suggesting that this could explain the decreased membrane degradation during ischemia.

The cardiac sarcoplasmic reticulum (CSR) is an important store of calcium for the activation of myocardial contraction. Relaxation occurs primarily by the energy dependent reuptake of calcium by the CSR. Fish oil supplementation caused a decrease in CSR function, and (n-3) PUFA were found to readily accumulate in CSR phospholipids (Taffet et al. 1993). Dietary (n-3) PUFA also modulate physico-chemical properties of sarcolemma by altering the fatty acid composition of the sarcolemma. The fish oil (n-3) PUFA, EPA and DHA, are taken up by the myocardium and incorporated into membrane phospholipids like phosphatidyl choline and phosphatidyl ethanolamine. This uptake is largely at the expense of arachidonic acid (Swanson and Kinsella 1986).

**Direct effects of fish oils on NEFA composition and the myocardium.** One early hypothesis suggested that after an acute myocardial infarction, arrhythmias were metabolically induced by acute lipid mobilization from adipose tissue which resulted in high free fatty acid (FFA) levels in the plasma and myocardial cells (Kurien and Oliver 1970). This excess FFA was believed to be arrhythmogenic and could increase the severity of ischemic damage. Under normal conditions tissue level of non-esterified fatty acid (NEFA or FFA) is very low. The majority of fatty acids are oxidized in the mitochondria to provide energy, and a small part is esterified and stored in the triacylglycerol and phospholipid pool (Van der Vusse et al. 1992). After meals glucose is the preferred fuel for myocardial oxidative metabolism, but during fasting NEFA becomes the preferred fuel. During ischemia when a part of the myocardium becomes anaerobic, fatty acid oxidation is disturbed, and unoxidized NEFA accumulate. This accumulation of NEFA is believed to be toxic to the heart and thought to stimulate arrhythmias (Oliver and Opie 1994). Riemersma et al. (1988) noted that there was an elevation in plasma NEFA in patients immediately after acute myocardial infarction and postulated that this could be a predisposing factor to the development of arrhythmias. Elevated levels of myocardial NEFA have also been observed in experimental animals subjected to ischemia.

Several mechanisms have been suggested as to how the increased NEFA levels may trigger arrhythmias. One hypothesis suggests that during ischemia,  $\beta$ -oxidation of lipids in mitochondria is inhibited and causes accumulation of intracellular acylcarnitine and acyl-CoA. This acylcarnitine in turn inhibits the Ca<sup>2+</sup> pump of the sarcoplasmic reticulum and calcium channels, causing an increase in Ca<sup>2+</sup> levels in the myocardial cells and thus causing arrhythmias (Corr et al. 1984, Huang et al. 1992). Another study lending support to this hypothesis reported the effects of increased NEFA levels on ventricular arrhythmias using ventricular fibrillation threshold (VFT) as an index of arrhythmogenicity (Makiguchi et al. 1991). They observed that the arrhythmogenicity of NEFA was due to a direct effect on the myocardial cells and due to the effect of NEFA esters such as long-chain acylcarnitine and acyl-CoA.

They speculated that the effect of NEFA was related to calcium overload in the myocardial cells.

It was also observed that during acute myocardial infarction, patients with excess saturated fatty acids (sum of 14:0, 16:0 and 18:0) in adipose tissues were most susceptible to ventricular arrhythmias (Abraham et al. 1989). The FFA hypothesis is considered controversial because in one study, despite the elevated plasma FFA levels after ischemia was induced in dogs, no increase in the incidence of arrhythmias was observed nor was VFT altered (Opie et al. 1971)

Another proposed mechanism suggests that altered proportions of AA, EPA or DHA in myocardial NEFA as a result of altered dietary fatty acids could lead to alterations in the NEFA pool that serves as immediate substrates for eicosanoid production. This could then lead to alterations in the production of myocardial TXA<sub>2</sub> and the vulnerability of the heart to develop arrhythmia during partial ischemia (Charnock et al. 1992b). That study demonstrated that after feeding a mixed diet relatively high in saturated fat and containing (n-3) PUFA, the NEFA had a direct effect on the heart by reducing the amount of eicosanoids and was one of the major factors that determined the vulnerability of the heart to develop VF, despite the very low NEFA concentration in the myocardial cell.

A study that looked at the effect of an intravenous infusion of (n-3) PUFA on ischemia-induced VF in exercising dogs (Billman et al. 1994) has once again renewed interest in this mechanism. Intravenous infusion of a fish oil emulsion containing EPA and DHA prior to inducing ischemia successfully prevented ischemia-induced VF. Since infusion was carried out 60 min before inducing ischemia, the possibility that the effect of fish oil was mediated via incorporation of (n-3) PUFA in sarcolemmal membranes was ruled out. The authors attributed the antiarrhythmic effects of fish oil to NEFA and postulated that unesterified PUFA, not saturated or monounsaturated fatty acids, prevented arrhythmias. In a recent study (Kang and Leaf 1996) of isolated myocytes, the authors have demonstrated that PUFA including AA, EPA and DHA can bind to the highly reactive sodium pump and prevent arrhythmias. They also found that since AA is rapidly converted to TXA<sub>2</sub>, which is proarrhythmic, only the (n-3) PUFA qualified for possessing antiarrhythmic properties. Saturated and monounsaturated fatty acids did not significantly bind to the Na<sup>+</sup> channel and were not believed to be antiarrhythmic, although LA was found to be mildly antiarrhythmic. They concluded that (n-3) PUFA did not have to be incorporated into membrane phospholipids because only free acids exhibited antiarrhythmic potential. In another recent study Weylandt et al. (1996) have shown that in cardiac myocytes cultured in NEFA-stripped medium supplemented with EPA and DHA, no antiarrhythmic effects were observed despite the membrane enrichment with EPA and DHA. This further substantiates the claim that (n-3) PUFA exert antiarrhythmic actions as free acids and not in phospholipids. But this in vitro system does not induce the release of NEFA as in an in vivo system where catecholamines and calcium activate phospholipase enzymes, which in turn trigger the release of NEFA following ischemia. The effects of different fatty acids on cardiac arrhythmia are summarized in **Figure 2**.

In our laboratory, the working hypothesis is that immediately following myocardial infarct NEFA are released from the hydrolysis of membrane phospholipids, and the type of NEFA released determines the arrhythmic response of the myocardium. Our working hypothesis thus compliments that postulated by Leaf and coworkers. However we believe that the incorporation of (n-3) PUFA into myocyte membrane is essential for antiarrhythmic action, so that these fatty acids are

readily available for release as free acids to prevent arrhythmias following myocardial ischaemia.

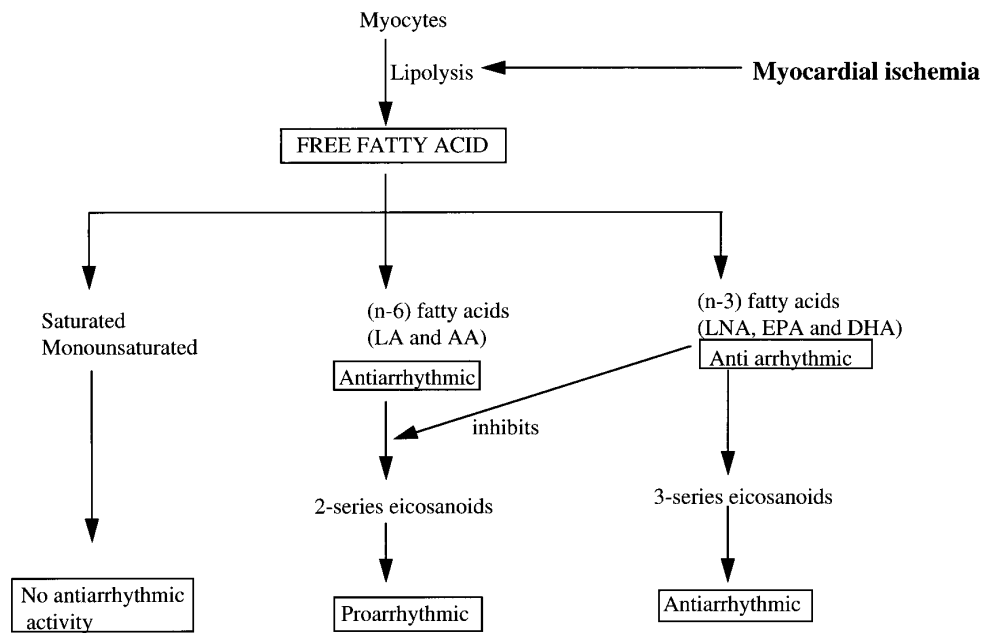
**Effect of (n-3) PUFA on the inositol lipid cycle and cell signalling.** An important aspect of membrane lipid metabolism in cells is the inositol lipid cycle. The inositol lipid cycle generates two important second messengers that are involved in cell signalling, namely phosphoinositides and diacylglycerol. An extracellular agonist interacts with a specific cell receptor to form the receptor/agonist complex (RAC) and initiates the inositol lipid cycle. The RAC activates a specific phospholipase C which then cleaves phosphatidyl inositols into two important second messengers, phosphoinositol triphosphate (IP<sub>3</sub>) and diacylglycerol. IP<sub>3</sub> activates the release of intracellular stores of calcium ions from the endoplasmic reticulum (sarcoplasmic reticulum) into the cytoplasm, facilitates entry of Ca<sup>2+</sup> ions into the cells and may alter electrophysiological properties of myocytes. The balance of Ca<sup>2+</sup> ions available for contraction is an important determinant of arrhythmias. Intracellular calcium concentration and disturbances of calcium homeostasis have been associated with arrhythmias (Manning and Hearse 1984). Alterations in cellular calcium can contribute to development of impulse generation and impulse conduction during myocardial ischemia (Billman and Leaf 1994). The major mode of entry of calcium into vascular smooth muscle is through two types of calcium channels, the voltage operated and the receptor operated channels (Opie 1991). In the voltage operated channel, intracellular calcium is released in response to the wave of electrical activity that spreads rapidly over the heart to initiate each contraction. In the receptor operated channel, TXA<sub>2</sub>, leukotriene B<sub>4</sub> (LTB<sub>4</sub>) and cytochrome P450 products of AA amplify an initial Ca<sup>2+</sup> related signal for cell activation by stimulating specific membrane receptors coupled to phospholipase C, thereby further increasing intracellular Ca<sup>2+</sup> concentrations (Weber 1990). (n-3)PUFA have been found to interfere at several sites in this signalling process.

Studies by Anderson et al. (1995) have shown that there is an increase in the release of IP<sub>3</sub> during reperfusion after acute ischemia. In a recent study by Jacobsen et al. (1996), the thrombin stimulated release of IP<sub>3</sub> during myocardial reperfusion was found to be greater than that observed in normoxic tissues. Their study suggests that thrombin is directly proarrhythmic, and the stimulation of IP<sub>3</sub> release initiated reperfusion arrhythmias.

**Effect of PUFA on calcium channels.** The effect of fish oil (n-3) PUFA on calcium has been found to be a secondary but powerful effect of one or more of the above mechanisms. Initial evidence for the role of dietary (n-3) PUFA in regulation of Ca<sup>2+</sup> release from the endoplasmic reticulum was provided by the studies of Kinsella et al. (1990) who demonstrated that there was an increase in Ca<sup>2+</sup> uptake by endoplasmic reticulum that was associated with the prevention of arrhythmias in rats raised on fish oil enriched diet.

Pioneering work by Hallaq et al. (1990, 1992) attempted to explain the effect of (n-3)PUFA on calcium channels. They demonstrated that DHA prevents toxic arrhythmias more effectively than EPA by modulating L-type calcium channels in the sarcolemma of cardiac myocytes, which prevents cytosolic free calcium levels from increasing to toxic levels. They also believe that the (n-3) PUFA are not merely acting as calcium channel blockers but as modulators, or valves, that control the influx and efflux of calcium to maintain normal contractility of the myocytes (Leaf 1988).

Hallaq et al. (1990) showed that the protective effect of (n-3) PUFA may not be due to the change in membrane phospholipids, but instead may be due to specific actions of (n-



**FIGURE 2** The effect of different dietary fatty acids on cardiac arrhythmia. LA, linoleic acid; AA, arachidonic acid; LNA, linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

3) PUFA and their metabolites. Ouabain inhibits the  $\text{Na}^+, \text{K}^+$ -ATPase pump and changes the electrophysiology of the myocardial cell causing sodium ions to accumulate. The increasing concentration of sodium ions in turn causes accumulation of calcium ions resulting in increased contraction and spontaneous beating rate in the myocytes. In myocytes incubated with AA there was no change after exposure to ouabain, while in myocytes incubated with EPA, ouabain toxicity was prevented. The authors suggest that EPA incorporated in membrane phospholipids, unlike AA, blocked ouabain binding which prevented the  $\text{Ca}^{2+}$  levels from increasing and thus preserved normal physiological levels of calcium. To further understand the role of calcium channels in the protective effect of (n-3)PUFA, the same group (Hallaq et al. 1992) studied isolated rat cardiac myocytes using nitrendipine, an inhibitor of the L-type calcium channel. This time they found that EPA protected the cardiac myocytes from ouabain toxicity by preventing the increase in free calcium to toxic levels via direct action on the calcium channels, not due to the incorporation of the (n-3)PUFA in membrane phospholipids. This study demonstrated that fish oil fatty acids appeared to exert a dual effect; they prevented excessive calcium influx but at the same time increased calcium influx when it was insufficient. **Figure 3** presents the link between the inositol lipid cycle and calcium levels and the possible effects in initiating arrhythmia within myocardial cells.

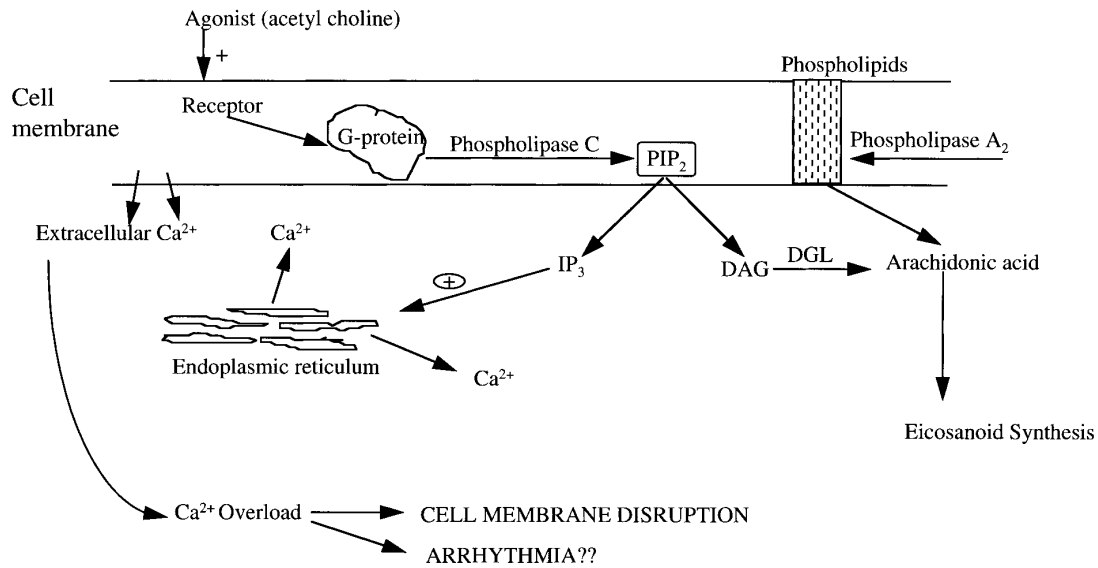
**Effect on enzymes.** Dietary (n-3)PUFA may influence the fluidity and the activity of enzymes required for energy production and of many lipid-protein dependent receptor functions (Charnock 1994). The activities of  $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase from sarcoplasmic reticulum, adenylyl cyclase and  $5'$ -nucleotidase are all markedly influenced by the levels of (n-6) and (n-3) PUFA in membrane lipids. In diets enhanced with fish oils rich in EPA and DHA, the activity of  $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase is decreased, adenylyl cyclase is increased and  $5'$ -nucleotidase is increased (Kinsella 1990).  $\text{Na}^+, \text{K}^+$ -ATPase and  $\text{Ca}^{2+}$ -ATPase play a significant role in the contraction and relaxation cycles of the cardiac muscle by maintaining ion levels within the myocytes (Vajreshwari and Narayanareddy 1992). Kinoshita

et al. (1994) found that EPA supplementation increased the activity of  $\text{Ca}^{2+}$ -ATPase within the myocardial cells, and this reduced the severity of the arrhythmias by inhibiting the  $\text{Ca}^{2+}$  accumulation following ischemia. Fish oil ingestion also affects cyclooxygenase enzyme activity, thus altering the pathways of eicosanoid metabolism (Knapp 1993).

Fish oil has been found to affect phospholipase enzyme activity. Activation of phospholipase  $\text{A}_2$  causes elevation of intracellular calcium. Phospholipase D activity is associated with the sarcolemma and is believed to be involved in second messenger signalling systems. Dai et al. (1995) reported that sarcolemmal phospholipase D is activated by unsaturated fatty acids like arachidonic and oleic acids.

**Effect on receptors.** Receptors in the sarcolemma are chiefly involved in the regulation of heart rate and contraction of the myofibers.  $\beta$ -Adrenergic receptors on the sarcolemma are involved in competitive inhibition by  $\beta$ -blockers or antagonists, the result of which can increase or decrease heart rate and contraction (Opie 1991). Studies by Grynberg et al. (1995) have looked at the effect of the two major fish oil fatty acids, EPA and DHA, on adrenergic receptor responsiveness. They found that a high DHA content in cardiomyocyte phospholipids appeared to decrease the  $\beta$ -adrenergic receptor affinity.

$\text{TXA}_2$  and prostacyclin, both derived from arachidonic acid, have potent but opposite actions on both vascular smooth muscles and platelets.  $\text{TXA}_2$  is a potent vasoconstrictor of arteries and promotes platelet aggregation, whereas prostacyclin is a vasodilator and inhibits platelet aggregation. Inhibitors and receptors have also been used to study the effect of thromboxane on arrhythmias. Studies in which UK38485, a specific inhibitor of thromboxane, was administered to greyhound dogs prior to occlusion of the left anterior descending artery (LAD) demonstrated that thromboxane is a major factor in the genesis of arrhythmias induced by acute myocardial infarction and reperfusion (Coker 1982). In another study by the same authors (Coker and Parrat 1985), intravenous injection of a thromboxane antagonist, AH23848, before and during acute myocardial ischemia was found to effectively block thromboxane receptors and to produce an antiarrhythmic ef-



**FIGURE 3** Inositol lipid cycle, cell signalling and calcium mobilization in myocardial cells. PIP<sub>2</sub>, phosphatidyl inositol 4,5 bisphosphate; IP<sub>3</sub>, inositol 1,4,5, triphosphate; DAG, diacylglycerol; DGL, diacylglycerol lipase.

fect. Blocking TXA<sub>2</sub>-endoperoxide receptors has also been shown to reduce ischemic injury to the myocardium (Hock et al. 1986).

Thrombin is the final enzyme in the procoagulant pathway and is an important regulator of various cellular events. One important role of thrombin is the activation of phospholipase C, which is involved in the generation of second messengers and the mobilization of calcium stores. A study of the effects of thrombin on the heart suggested that it might prolong repolarization and cause electrical abnormalities which could lead to ischemia (Steinberg et al. 1991). The effect of diet or fish oil supplementation on thrombin has not been studied, but this could be a possible mechanism and warrants further investigation.

## CONCLUSION

All the studies reviewed, both in animals and in the human intervention trials, leave little doubt that dietary supplementation of fish oils confers beneficial effects on the heart particularly in the prevention of cardiac arrhythmias. But no definite mechanism has yet been implicated. All the mechanisms suggested appear to be interrelated, and it is not known whether their effects are independent or are compounded and in what sequence they take place. It is also not clear whether the mechanisms by which (n-3) PUFA prevents cardiac arrhythmia are mediated via changes in plasma and/or vascular tissues or if they directly affect the electrophysiology of the cardiomyocytes. The former hypothesis is supported by antiarrhythmic studies of diet supplemented animals while studies using isolated animal heart and cultured neonatal cardiomyocytes tend to support the latter hypothesis. The (n-3) PUFA from fish oils have also been found to modify several other factors like enzymes and receptors, and their involvement in antiarrhythmic mechanisms is also being investigated. Our lab is currently involved in a series of experiments using pigs as a model to study the antiarrhythmic mechanism of fish oils. Previous studies have examined the effects of diets high in (n-3) fatty acids in smaller animals which do not allow detailed electrophysiological study. Pigs have a similar cardiac physiology and coronary anatomy to humans. And more importantly, the pigs have

a fatty acid metabolism (Chapman 1980) similar to humans which makes it a suitable model to study these mechanisms in detail.

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