Chapter 4. Discussion

Through this evidence review, we have examined whole animal studies, whole animal isolated organ and cell studies, and isolated organ and cell culture studies to determine the effects of omega-3 fatty acids on arrhythmogenic outcomes and on myocardial cell organelles involved in cardiac electrogenesis. In this chapter, we discuss main findings from the studies and highlight study limitations and opportunities for future research. Findings from whole animal studies are discussed first, followed by whole animal isolated organ and cell studies and isolated organ and cell culture studies.

Whole Animal Studies

Based on the meta-analyses of the incidence of total deaths, ventricular tachycardia, and ventricular fibrillation in ischemia- and/or reperfusion-induced arrhythmias, we conclude that fish oil supplementation has anti-arrhythmic effects in the rat model when compared to omega-6-fatty acid supplementation. Our findings are summarized in the following table:

Table 4.1 Comparisons of fish-oil to Omega-6 supplementation

Experiment Conditions	Outcomes	Animal models	Omega-3 Arms	Doses of EPA+DHA (g/100 g)	# Comparisons [# Studies]	# Animals	Combined RR [a] (95% CI)
Ischemia reperfusion-induced arrhythmias	Incidence of total deaths	Rats	ALA oils	0.4 - 1.2	5 [2]	133	1.2 (0.51-2.6)
			Fish oils	1.1 - 3.7	7 [6]	169	0.47 [b] (0.23-0.93)
Ischemia-induced arrhythmias	Incidence of ventricular tachy-cardia	Rats	ALA oils	0.4 - 5.2	4 [3]	112	0.82 (0.65-1.0)
			Fish oils	2.1 - 3.7	6 [6]	136	0.49 (0.29-0.83)
Ischemia-induced arrhythmias	Incidence of ventricular fibrillation	Rats	ALA oils	1.1 - 5.2	3 [2]	76	0.95 (0.56-1.6)
			Fish oils	2.1 - 3.7	5 [5]	100	0.21 (0.07-0.63)
Reperfusion-induced arrhythmias	Incidence of ventricular tachy-cardia	Rats	ALA oils	0.4 - 1.2	5 [2]	125	1.1 (0.73-1.6)
			Fish oils	2.6 - 3.7	6 [5]	132	0.68 (0.50-0.91)
Reperfusion-induced arrhythmias	Incidence of ventricular fibrillation	Rats	ALA oils	0.4 - 5.2	6 [3]	144	0.84 (0.52-1.3)
			Fish oils	1.2 - 3.7	8 [7]	168	0.44 (0.25-0.79)

[a] Random-effect model
[b] The significantly reduced risk ration of deaths was due to a single study. After removing the study, the combined risk ratio of deaths became 0.64 (0.19-2.1)
g= grams

Fish oil supplementation in rats showed significant protective effects for ischemia- and reperfusion-induced arrhythmias by reducing the incidence of ventricular tachycardia and fibrillation. The anti-arrhythmic effects seemed stronger in ischemia-induced arrhythmias than in reperfusion-induced arrhythmias. No beneficial effects related to ischemia- and/or reperfusion-induced arrhythmias were found for alpha linolenic acid (ALA 18:3 n-3) supplementation in the rat model when compared to omega-6-fatty acid supplementation (Table 4-1). Results were consistent in the 2 studies directly comparing the anti-arrhythmic effects of ALA oils to fish oils. The incidence of total deaths, ventricular tachycardia, and ventricular fibrillation were lower in rats fed fish oil than in rats fed soybean or linseed oils (Table 3-11).

In monkey models, fish oil supplementation was found to prevent deaths in ischemia- and isoproterenol-induced arrhythmias in one study (Table 3-4). In addition, 3 studies examined ventricular fibrillation threshold and the incidence of ventricular fibrillation in induced arrhythmias. No anti-arrhythmic effects were seen in normal and ischemic conditions. There was a non-significant reduction in the incidence of ventricular fibrillation, and an increase in ventricular fibrillation threshold, in isoproterenol-induced arrhythmias among monkeys fed fish oils compared to monkeys fed sunflower seed oil (Table 3-9).

One study compared hypertensive rats fed EPA, DHA, or a mixture of EPA plus DHA, to rats fed monounsaturated fatty acid. This study showed a significantly reduced incidence of ventricular fibrillation in rats fed DHA or EPA plus DHA, but no significant reduction in rats fed EPA alone [29].

In contrast to studies of rats fed saturated fatty acids, 5 studies showed consistent protective effects on ischemia- and/or reperfusion- induced arrhythmias in rats, rabbits or pigs fed fish oils, although again the results were not statistically significant for most comparisons (Table 3-12 to Table 3-15). Similar results were found in 4 studies that compared dogs fed fish oil or EPA esters to no treatment controls (Table 3-16 to Table 3-17).

Summarizing the results from studies that compared pre-fed fish oil to pre-fed omega-6 fatty acids, monounsaturated fatty acids, saturated fatty acids, or no treatment controls across various species (rats, monkeys, dogs, rabbits, and pigs), we conclude that fish oil supplementation might have anti-arrhythmic effects when compared to omega-6 or monounsaturated fatty-acid supplementation. The anti-arrhythmic effects were apparent when animals fed fish oil were compared with those fed saturated fatty acids or with no treatment controls. In most of the studies that showed a non-significant reduction in the incidence of death, ventricular tachycardia, and ventricular fibrillation, the lack of significance was likely due to lack of statistical power. Only one study [35] reached the minimum group size to detect a 50% reduction in arrhythmic effects, as shown in Table 4.2:

Table 4.2 Minimum group size to detect a 50% reduction in ventricular fibrillation

Control group incidence in ventricular fibrillation	Group size* (N)
90	14
80	20
70	28
60	40
50	73
40	100

* Assuming two equal groups, a power of 80% to show the arbitrarily selected "physiologically" significant effect at P=.05 Adapted from Riemersma et al. [102].

A total of 3 infusion studies were found. Two studies, both by the same author, reported "acute" anti-arrhythmic effects for albumin-bound ALA, EPA plus DHA, and fish oil emulsion in the dog model [41,42]. However, the study author was concerned about potential toxic effects of intravenously infused fish oil emulsion and discussed an example in which 10 g of albumin would expand the intravascular volume acutely by some 20% in a 20-kg dog, which might induce acute congestive heart failure. The other study found ALA emulsions increased ventricular premature beats and ventricular tachycardia in dogs (Table 3-18). The mechanisms of the observed anti-arrhythmic effects of albumin-bound ALA, EPA plus DHA, or fish oil emulsion are still unknown. Therefore, we conclude that the arrhythmic effects for albumin-bound ALA, EPA, DHA, and, fish oil emulsion is unknown. Also, there is some concern about potential toxic effects of the emulsions.

Study Quality

In human clinical trials, randomization, allocation concealment, blinding of investigators and subjects, and adequate sample size are recognized as key factors that might affect the quality of the study and reliability of the study results. Most of these factors could be implemented in whole animal studies, but might not be relevant to cell culture studies. A series of guidelines for the study of arrhythmias in ischemia, infarction, and reperfusion provide some insights on the quality of whole animal studies included in this review [14]. Following is a summary of their conclusions for how to conduct research on the mechanisms of arrhythmias in animal studies:

- Randomization of treatment and blinded analysis are essential.
- No species or model is ideal. All species and models have their limitations. Thus, multiple species and models should be sought.
- Comprehensive background information on animals must be reported. This should include the animal source, strain, sex, age, body weight, housing condition (diet, light/dark cycle, number of animals per cage), and experimental environment (ambient temperature, time of day, and season).
- Controls should be contemporary and preferably be equal in group size to the intervention groups.
- Exclusion criteria must be determined before the start of an experiment, stated explicitly, and applied in a blind manner. Animals excluded from a study, and the reasons for their exclusion, must be reported.
- Treatments (e.g., the compositions of experimental and control diets) and outcome measures should be clearly defined and reported. Experimental models should be independent of each other.

Of the 26 whole animal studies, only 3 studies explicitly reported the randomization to treatment, and no study reported blinded analyses. Animal characteristics and housing conditions were described in most studies; however, cross-referencing to the prior papers is common.

Contemporary controls were used in all but monkey and infusion studies. Exclusion criteria were rarely used.

Limitations and Future Research

Because meta-analysis is based on published studies, it is limited by its observational design. In order to increase statistical power, our meta-analyses combined the same species of animal but different strains (eg. Wistar rats and Sprague-Dawley rats) across different age groups of animals. This could introduce "noise" for the observed effects. Although the random-effects model takes the variability between studies into account, the relative risks of the arrhythmic outcomes are based on summary statistics without access to primary data of individual studies. The observed effects from meta-analyses were therefore not adjusted for other factors that could affect the outcomes, such as the amount of saturated, monounsaturated, or omega-6 fatty acids in animals' diets. We tried to minimize the confounding factors by choosing the optimal comparison from each study (Methods section), so that all comparisons in the meta-analyses were iso-caloric and had minimum differences in the fatty-acid compositions in the diets. However, the fatty-acid compositions in the diets were not totally controlled due to different sources of added fats between groups. These factors could be adjusted using a statistical method developed by Fay et al. [103], but the subjects included in the analyses should be homogeneous except for differences in the controlled factors. This method has been used in a meta-analysis on the effect of different types and amounts of fat on the development of mammary tumors in rodents [104,105].

Even though 26 whole animal studies were identified, about 70% of studies included in the meta-analyses are from the same group of collaborating researchers, such as M. Abeywardena, J. Charnock, and P. McLennan. This is one of the reasons for the standardization of arrhythmic outcome measures. The results reported from a single laboratory should be independently verified by another. More research from various laboratories on potential mechanisms for the effects of omega-3 fatty acids on arrhythmia is needed.

Whole-Animal/Isolated Organ and Cell Studies

Contractile Parameters

Eight studies evaluated the effect of diets enriched with omega-3 fatty acids on various contractility parameters. Four studies examined the impact of omega-3 fatty acids on heart rate. No definitive conclusion can be drawn from these studies due to the limited number of studies and because they each compared different diets and used different experimental conditions. Only 4 studies (2 by the same author) examined the effect of omega-3 diets on contractility parameters, and these studies produced conflicting results. Therefore, definitive conclusions cannot be drawn. There were only 2 studies of inotropic effects, and their use of different inotropic parameters precludes comparison. No inference regarding cardiac work is possible, since only one study examined the impact of linseed oil on this inotropic parameter.

Basoelectromechanical Parameters

Only 3 studies evaluated the effect of diets enriched with omega-3 fatty acids on basoelectromechanical parameters. No strong inference is possible because of the small number of studies and the inconsistent results across the species studied (rats and rabbits).

Ion Pumps and Ion Movement

Fourteen studies examined the impact of omega-3 fatty acid diets on IPIM. Of the 8 studies that examined pump activity, 5 addressed calcium-magnesium ATPase activity. 3 of these were from the same laboratory. The results of the 3 related studies varied with the type of omega-3 fatty acid used and the comparison group. The other 2 studies showed an increase in calcium-magnesium ATPase activity. In contrast, there was consistency among results from 3 independent groups that studied sodium-potassium ATPase activity, with each of these groups finding no change attributable to omega-3 fatty acids. Inferences about cytosolic calcium influx are limited by the small number of studies and the use of different comparison groups. Similarly, there was only a single study of cytosolic calcium efflux. There were only 3 independent studies of cytosolic calcium content (with one examining the effect of animal age), but they were consistent in observing no change in this parameter. However, under ischemic-reperfusion conditions, results were contradictory.

Two of 3 studies examining the effect of fish oil diet supplementation on sarcoplasmic reticulum calcium content showed a decrease, with the other reporting no change in this parameter. Inferences are limited, however, due to the small number of studies. There was only one study of sarcoplasmic reticulum calcium release, 1 of sarcoplasmic reticulum calcium transport activity, 1 of the sarcoplasmic reticulum calcium exchanger or efflux,and 2 of sarcoplasmic reticulum calcium uptake. Although the latter 2 showed consistent decreases in uptake, the small number of studies limits inference.

Ion Currents

Only 2 studies examined the effect of omega-3 fatty acid dietary supplementation on ion currents. One of the studies examined sodium current (I_{Na}) and transient outward potassium current (I_{to}), and the other measured voltage dependent L-type calcium current . No change was observed in these parameters, but no inferences can be made due to the small numbers of studies.

Ion Channels

There were only 2 studies of ion channels. Although both examined the effect of an omega-3 fatty acid enriched diet on the calcium channel, they examined different parameters and are therefore not comparable.

Summary of Areas for Future Research

Table 4.3 summarizes areas for future research in whole animal and isolated organ and cell culture studies by showing the fatty acids tested, the number of studies of each parameter, and a

rough assessment of the degree of consistency of study results. In general, it shows that there were small numbers of studies for most parameters and inconsistent results, as well as areas where no studies at all were identified.

Isolated Organ and Cell Culture Studies

Contractile Parameters and Arrhythmias

All 7 studies of arrhythmia in isolated organs and cell cultures showed that omega-3 fatty acids (predominantly EPA and DHA, but in one instance ALA) appear to have a protective effect against spontaneous or induced arrhythmias in both rat and guinea pig models. However, it must be noted that 4 of the 7 studies were from the same collaborative group. Additionally, one study [69] seemed to indicate that the omega-3 fatty acids must be in the free and not the bound form (we termed the former 'free' fatty acids and the latter as 'bound' fatty acids) to exert its protective effect, but this finding was contradicted by another study which observed a significant decrease in the proportion of induced arrhythmias with bound DHA. In the guinea pig model, there appears to be a dosage

TABLE 4.3 Areas for future research: Whole animal and isolated organ and cell culture studies

Outcome Variable	# of Studies Identified	Fatty Acid Tested				Results[a]		
		FO	EPA	DHA	ALA	NC	I	D
Contractile and Arrhythmogenic Parameters								
Heart Rate	4	x	x	x	-	x	-	x
Contraction Rate	4	x	-	-	-	x	x	x
Ionotropic Parameters	2	x	-	-	-	x	-	x
Cardiac Work	1	-	-	-	x	x	-	-
Basoelectromechanical Parameters								
Developed or Resting Tension	1	x	-	-	-	x	-	-
Other parameters [b]	2	x	-	-	-	x	-	x
Ion Pumps and Ion Movement								
Pump Activity	8	x	x	x	-	x	x	x
Cytosolic Calcium Influx	2	x	-	-	-	x	x	-
Cytosolic Calcium Efflux	1	x	-	-	-	x	-	-
Cytosolic Calcium Content	3	x	-	-	-	x	-	x
Sarcoplasmic Reticulum Calcium Content	3	x	x	x	x	x	-	x
Sarcoplasmic Reticulum Calcium Uptake	2	x	-	-	-	-	-	x
Sarcoplasmic Reticulum Calcium Release	0	-	-	-	-	-	-	-
Sarcoplasmic Reticulum Calcium Exchanger	1	x	-	-	-	-	x	-
Ion Currents								
Sodium Current	1	x	-	-	-	x	-	-
Transient Outward Potassium Current	1	x	-	-	-	x	-	-
Voltage Dependent L-Type Calcium Current	1	x	-	-	-	x	-	-
Delayed Rectifier Potassium Current	0	-	-	-	-	-	-	-
Inward Rectifier Potassium Current	0	-	-	-	-	-	-	-
Ultra Rapid Potassium Current	0	-	-	-	-	-	-	-
Ion Channels								
Binding to the Calcium Channel	2	x	-	-	-	x	-	x

[a] NC=no change; D=decrease; I=increase

[b] VERP (Left ventricular effective refractory period), ARP (Functional refractory period of the atrium), RRP (Relative refractory period), QRS (Ventricular conductance time), QT, MAP (monophasic action potential duration)

'-' indicates no studies; 'x' indicates at least one study

Note: This table does not include results from studies that compared young versus aged animals or different doses of omega-3 fatty acids.

threshold at which EPA can exert its protective effect. Whether this also applies to ALA and DHA needs to be verified.

The sub-category of contractility changes had the largest number of studies, and the results were consistent. In the presence of various arrhythmogenic agents and across the different types of species studied, omega-3 fatty acids compared to controls were reported to consistently decrease contraction rate, thereby exerting a protective effect with respect to arrhythmia. In studies without an arrhythmogenic agent, the results were, however, inconsistent, with 3 showing a decrease in contractility and 3 showing no effect.

Only one study examined the effect of DHA vs. a saturated fatty acid and showed a decrease in contractility with DHA. Further research is needed to determine whether other omega-3 fatty acids would have the same effect. Similarly, only 2 studies compared the effect of an omega-3 fatty acid vs. an omega-6 fatty acid and the results were inconsistent, again suggesting the need for further research. Only 2 studies by the same author compared the relative efficacy of one omega-3 fatty acid to another omega-3 fatty acid, and the results suggested that EPA is more effective than DHA in reducing the frequency of spontaneous contractions. We found no other studies that validated these findings.

Two studies showed that methylated or ethylated omega-3 fatty acids had no effect on contractility, suggesting that a free carboxyl group is necessary for omega-3 fatty acids to exert their anti-arrhythmogenic effect. This finding was, however, contradicted by another study, again suggesting the need for more research. Two studies examined the effect of omega-3 fatty acids on twitch size, but the results were inconsistent. One study reported a dose dependent effect, but again the small number of studies suggests the need for further research. The identification of only 3 studies of inotropic parameters with conflicting results limited inference about these parameters.

Of the 7 studies examining other contractility parameters (tC_{20}, CD_{20}, CD_{80}, -Cmax, and +Cmax), 2 examined the effect of omega-3 fatty acids relative to omega-6 fatty acids under ambient, hypoxic, and reoxygenation conditions and showed no difference in effect across agents. Four of 5 compared EPA to DHA and found no difference in effect. Despite the consistency of these findings, the omega-3 fatty acids were all in the bound form. We found no studies that addressed whether the results would be similar if the omega-3 fatty acids were in the free form.

Basoelectromechanical Parameters

The 6 studies that measured action potential had widely varying study designs. They used different agents and experimental conditions as well as different comparison groups. The results were inconsistent. Therefore, despite the relatively large number of studies, no inferences can be made and further research is needed. There were 7 studies of the effect of omega-3 fatty acids on action potential amplitude. Again, these studies used different comparison groups and different experimental conditions. These results were also inconsistent, limiting the conclusions that can be drawn.

Only 4 studies (3 of them from the same group of collaborators) examined action potential duration at 40% polarization. The studies were carried out under a variety of experimental conditions, and the results varied considerably, with some investigators reporting increases and others reporting decreases in the duration of the action potential. Among the studies of action potential duration at 80% polarization, a decrease or no change was more frequently reported than

an increase, but the small number of studies (4 from the same collaborators) and varied experimental conditions limit inference.

There were 6 studies (4 of them from the same group of collaborators) that examined the effect of omega-3 fatty acids on maximum rate of depolarization (V_{MAX}). The results varied depending on the experimental condition, thus limiting inference.

There were 4 studies (all from the same collaborative group) that examined the effect of omega-3 fatty acids on maximum diastolic potential. The predominant finding was that there was no impact on this parameter, but the results varied depending on the comparison group and experimental condition under which the studies were performed, thereby precluding inference.

There were 4 studies (all from the same collaborative group) that measured the overshoot potential following omega-3 fatty acid treatment. Since the results varied depending on the comparison group and experimental condition under which the studies were performed, no inference can be drawn.

Ion Pumps and Ion Movements

Only 1 study examined the impact of bound EPA on pump activity (sodium-potassium ATPase). This study reported no effect in the presence of ouabain (OUA) or bumetanide (BUME), or with a combination of these 2 agents [72].

There were only 3 studies (2 from the same group of collaborators) that examined the impact of omega-3 fatty acids on cytosolic calcium influx. These studies consistently reported that omega-3 fatty acids were effective in preventing increases or decreases in cytosolic calcium influx induced by various agents. There was only 1 study of cytosolic calcium efflux, so no inference can be drawn. Two studies from the same collaborators found that cytosolic calcium content decreased in the presence of free EPA. Similarly, 2 studies from another pair of collaborators showed a protective effect of free or bound DHA or EPA in the presence of various agents that altered cytosolic calcium content. One of these researchers also found that acute exposure to free DHA had a smaller effect than chronic exposure.

The identification of only 1 study of sarcoplasmic reticulum calcium content precludes inference. We identified no studies that examined the effect of omega-3 fatty acids on sarcoplasmic reticulum uptake of calcium. Two studies evaluated sarcoplasmic reticulum calcium release, and the findings were inconsistent.

Two studies in dogs by the same investigator showed that ALA increased sodium-calcium exchange. The effect of EPA or DHA on this parameter is unknown. Another study using a guinea pig model measured sodium-hydrogen exchange and showed no effect with ALA but a dose dependent decrease with EPA and DHA. Further research is needed in this area.

Ion Currents

Three studies examined the effect of omega-3 fatty acids on sodium current (I_{Na}), and 2 showed a decrease. With regard to the activation and inactivation parameters of the sodium current, the results are contradictory; thus, no conclusions can be inferred from these studies. With the exception of 1 study which showed an increase in amplitude of the I_{to}, 3 studies (2 rat models and 1 ferret) showed a decrease in amplitude. More research is required to verify this finding. With the exception of 1 study using a guinea pig model, and another using various agents, the remaining

4 studies reported a decrease in voltage dependent L-type calcium current ($I_{Ca.L}$), and this observation was consistent for all omega-3 fatty acids tested as well as across species.

Two studies both showed a decrease in delayed rectifier potassium current (I_K), but more studies are needed to support this conclusion. Out of the 4 studies of inward rectifier potassium current (I_{KI}), 1 showed a decrease, while the other 3 showed no effect for any omega-3 fatty acid tested using either a mouse, rat, or ferret model. No strong inferences can be made regarding ultra rapid potassium current (I_{KUR}) because only 2 studies were found. Both of these, however, showed a decrease in I_{KUR} when fatty acid concentrations were above 20µM.

Ion Channels

Only 3 studies (2 from the same group of collaborators) examined the effect of omega-3 fatty acids on ion channels. Because each study examined different parameters, no conclusions can be inferred from these studies.

Summary of Areas for Future Research

The following table summarizes areas for future research in isolated organ and cell culture studies by showing the fatty acids tested, the number of studies of each parameter, and a rough picture of the degree of consistency of study results. In general, it shows that there were small numbers of studies for most parameters and inconsistent results, as well as areas where no studies at all were identified.

Study Design and Analysis Issues: Isolated Organ and Cell Culture Studies

A number of issues presented challenges to the synthesis of data on the effects of omega-3 fatty acids on arrhythmogenic mechanisms in isolated organ and cell culture studies. Examples of each are discussed below.

General Design Issues

Sample sizes were sometimes not reported or often difficult to ascertain. Often, studies presented results only graphically, precluding quantitative analyses of the results. In these instances, supplementary tabular presentations would add to the usefulness of the research.

Sub-Grouping Based on Multiple Interventions and End Points

Within each of the sub-areas studied — ion channels, ion currents, ion pumps and ion movement, and contractility — there were numerous sub-parameters. For example, arrhythmogenic and contractile parameters included more than 8 sub-parameters. There are many potential variables by which it would be instructive to subgroup when analyzing these types of data, including species (at least 4 types), fatty acid (at least 4 types), form of fatty acids

(free/bound), age of the animal (young vs. old), dosage (at least 2 levels), agents (antagonists, agonists, etc.), and conditions (ambient, etc.). Sub-grouping in systematic reviews is often challenging given that there are many potential confounding variables. Our response to this reporting challenge was to focus on the three most important sub-grouping variables: species, fatty acid type, and form of the fatty acid.

Additionally, the large number of sub-measures posed a challenge. It would therefore benefit the field to identify core sets of standardized measures that produce the highest information yield and to encourage investigators to include at least these measures in future studies. The practice by various investigators of frequently choosing different measures greatly reduces the options for synthesizing results across different studies. If there were large numbers of studies for each

Table 4.4 Areas for future research: Isolated organ and cell culture studies

Outcome Variable	# of Studies Identified	Fatty Acid Tested				Results[a]		
		FO/ Combo	EPA	DHA	ALA	NC	I	D
Contractile and Arrhythmogenic Parameters								
Spontaneous or Induced Arrhythmia	8	-	x	x	-	-	-	x
Contractility	18	x	x	x	x	x	-	x
Ionotropic Parameters	3	-	x	x	-	x	x	-
Other Contractility Parameters*	7	x	x	x	-	x	-	-
Basoelectromechanical Parameters								
Action Potential	6	x	x	x	-	x	x	x
Action Potential Amplitude	7	-	x	x	-	x	x	x
Action Potential Duration at 40% Depolarization	4	x	x	-	-	x	x	x
Action Potential Duration at 80% Depolarization	6	x	x	x	-	x	x	x
Maximum Rate of Depolarization	5	x	x	-	-	x	x	x
Maximum Diastolic Potential	3	x	x	-	-	x	-	-
Overshoot Potential	4	x	x	-	-	x	x	x
Other #	1	-	-	-	x	x	-	-
Ion Pumps and Ion Movement								
Pump Activity	1	-	x	-	-	x	-	-
Cytosolic Calcium Influx	3	-	x	x	-	x	-	x
Cytosolic Calcium Efflux	1	-	x	x	-	x	-	-
Cytosolic Calcium Content	7	-	x	x	-	x	x	x
Sarcoplasmic Reticulum Calcium Content	1	-	x	x	-	-	x	-
Sarcoplasmic Reticulum Calcium Uptake	0	-	-	-	-	-	-	-
Sarcoplasmic Reticulum Calcium Release	2	-	x	x	-	-	x	x
Sodium-Calcium Exchangers	1	-	-	-	x	-	x	-
Sodium-Hydrogen Exchangers	1	-	x	x	x	x	-	x
Calcium transients	1	-	x	-	-	-	-	x
Passive SR calcium efflux	2	-	-	-	x	-	-	x
Passive sodium influx	1	-	x	x	-	x	-	-
Ion Currents								
Sodium Current	3	-	x	x	x	x	-	x
Transient Outward Potassium Current	3	-	x	x	-	-	x	x
Voltage Dependent L-Type Calcium Current	6	-	x	x	x	x	x	x
Delayed Rectifier Potassium Current	2	-	x	x	x	-	-	x
Inward Rectifier Potassium Current	4	-	x	x	x	x	-	x
Ultra Rapid Potassium Current	2	-	x	x	-	x	-	x
Ion Channels								
Sodium Channel	1	-	x	-	-	x	-	-
Cloned Kv1.5 Potassium Channel	1	-	-	x	x	x	-	x
Calcium Channel	1	-	x	-	-	x	-	-

[a] NC=no change; D=decrease; I=increase

* tC20, CD20, CD80,-Cmax, +Cmax

AC (Intra-atrial conduction time), AVC (Atrioventricular conductance time), ARP (Functional refractory period of the atrium), AVRP (Functional refractory period of atrio-ventricular conducting system)
'-' indicates no studies; 'x' indicates at least one study

Note: This table does not include results from studies that compared young versus aged animals or different doses of omega-3 fatty acids.

parameter of interest, the challenge would not be so daunting. The number of articles for each sub-parameter, however, is often quite limited, so robust inference is frequently precluded. We grouped outcomes reported in the various studies into 5 major categories — contractile parameters (CP), basoelectromechanical parameters (BEP), ion pumps and channels (IPIM), ion currents (ICU), and ion channels (ICH) — to aid in the summary of results. However, we found a wide variation in reports of the same outcome due to different experimental methods. Thus, there is a need for researchers to limit the number of outcomes reported and to reach a consensus on which outcomes are the most relevant and standardizable. For example, contractility parameters such as contraction coupling delay (tC_{20}), contraction duration at 20% relaxation (CD_{20}), contraction duration at 80% relaxation (CD_{80}), relaxation time (-Cmax), and cell shortening velocity (+Cmax) were almost always reported in studies of arrhythmia.

In Vitro Models

Tissues or cells from various species of animals, including mice, rats, guinea pigs, ferrets, dogs, pigs, and cats, were used to examine the effect of omega-3 fatty acids on arrhythmogenic mechanisms. However, upon reviewing the data, it appears that the results reported are not always applicable across species, all cardiac cell types used (atrial, ventricular, etc.) and all development stages (neonatal, adult). Thus, it might be prudent to reach a consensus on the animal model or models whose basic cardiac physiology, biochemistry, and fatty acid metabolism are as similar as possible to human cardiac tissue, and then for the various research groups to use these models to conduct their experiments. For example, to study arrhythmogenic outcomes, cultured neonatal rat cardiomyocytes appear to have certain advantages over other models: they beat spontaneously at rates that can be monitored, they are robust and capable of surviving for several days thereby allowing for incorporation of the omega-3 fatty acids into membrane phospholipids, and they provide a system free of neuronal or hormonal influences. However, to determine the effect of omega-3 fatty acids on ion pumps or channels (e.g. sarcoplasmic reticulum calcium ATPase, cellular calcium flux), the rabbit, ferret, cat, dog, and guinea pig models more closely mimic humans compared to the rat or mouse models. Recently, some investigators appear to be using transgenic cardiac tissue with cloned human ion channels, human embryonic kidney cells, etc. to determine the arrhythmogenic mechanisms of omega-3 fatty acid effects which might be more relevant to the human situation. This needs further investigation.

Exposure Duration

In our review of the data, we found that some investigators chose to examine the effects of the omega-3 fatty acids by directly adding them to the culture medium, or incubated the cells with the omega-3 fatty acid to allow for incorporation into membrane phospholipid. We termed the former as "free" fatty acids and the latter as "bound" fatty acids. It appears that some investigators feel that the omega-3 fatty acids exert their effect only in the free form, and this is supported by 2 studies

[69,78]. However, both of these studies were from the same research group. To further substantiate their claim, the researchers added delipidated bovine serum albumin (BSA) to remove the omega-3 fatty acids from the culture medium and showed a reversal of the protective effect. Thus, they concluded that the fatty acids do not form strong covalent or ionic bonds with any constituent of the cell membrane, but rather act directly by partitioning into the hydrophobic interior of the plasma membrane phospholipids. Other investigators feel that the incorporation of the omega-3 fatty acids into the cardiomyocyte membrane is essential for its antiarrhythmogenic effect and, indeed, this is supported by the clinical and whole animal feeding studies. Nair [5] notes, "that following a myocardial infarct, non-esterified free fatty acids (NEFA) are released by hydrolysis from the membrane phospholipid, and the type of fatty acid released determines the arrhythmogenic response of the myocardium." He notes that this would support the free fatty acid hypothesis, but adds, "the omega-3 fatty acids would first have to be incorporated into the membrane phospholipid to be available for release as free acids to prevent arrhythmias following myocardial ischemia."

Amount of Omega-3 Fatty Acid Used

We found that the concentration of omega-3 fatty acids used in the various studies were markedly different, ranging from 1µM to 214µM. The results obtained at concentrations greater than 20µM are questionable due to non-specific effects such as detergent effects on ion channels, etc. While some studies have attempted to quantify IC50 (that concentration that produces a 50% reduction in the effect) values (Table 3-31), the results are inconsistent which might reflect the purity of the omega-3 fatty acid, solvent used (ethanol etc.), the transport agent, or the form (sodium salt, methylated or ethylated omega-3 fatty acid). In fact, 3 independent studies have shown that compared to EPA or DHA, methylated DHA ester or ethylated EPA ester do not exert the same protective effect. One study contradicts this finding. Thus, there is a need to develop standard preparations of omega-3 fatty acids (e.g. both as free fatty acid and triacylglycerol) that would be available from the National Institutes of Health (NIH) or other suppliers to all researchers with a valid protocol. Additionally, a consensus needs to be reached on the omega-3 dosage. Addressing these issues is critical for interpreting the relevance of data from isolated organ and cell culture studies to humans. This is particularly true for data regarding the dietary and supplemental intake and the metabolic processing of omega-3 fatty acids.

Comparison Group

While a majority of the studies reported results compared to a control, it might be more relevant to use an omega-6 or monounsaturated fatty acid as the comparison group (see section on Diet Classification section in Chapter 2). Additionally, only 3 studies evaluated the effect of one omega-3 fatty acid to another omega-3 fatty acid. This area needs further research.

Experimental Condition or Agent

The most challenging task was to classify studies based on experimental condition and agent used. We identified 3 conditions (ambient, hypoxia, reoxygenation). Unfortunately, the results obtained under these conditions seemed to be very inconsistent. Additionally, the number of

agents within and across studies varied considerably. While the effect of omega-3 fatty acids in the presence of agents such as indomethacin and nitrendipine help answer the question as to whether the action of the omega-3 fatty acids is exerted via their metabolites and sites of action, the use of numerous other arrhythmogenic agents (e.g. BAY8644) seems excessive and clinically irrelevant. It might be appropriate to convene an expert panel to evaluate and standardize available methods (ischemic models vs. arrhythmogenic models) that is more relevant to the human situation so that the results are comparable across studies and are more applicable or generalizable.

IC50 and EC50 Values

Four studies (2 by the same author) reported omega-3 fatty acid IC50 or EC50 (that concentration needed to produce a 50% effect) values for I_{Na}, I_{to}, $I_{Ca.L}$, I_K, and twitch size (TS). For I_{Na}, 1 study showed in a rat model that DHA was more effective than EPA, which was more effective than ALA, in decreasing I_{Na} [97]. This was not supported by the results of another study which showed that EPA was more effective than DHA in both rat and guinea pig models [83]. For I_{to} and $I_{Ca.L}$, one study showed that EPA was more effective than DHA in both rat and guinea pig models [83]. That study also suggested that EPA was more effective in the rat model in decreasing $I_{Ca.L}$. See Table [3-31].

Conclusion

In studies using whole animal and whole animal isolated organs and cells, the question regarding plausible biochemical or physiological mechanisms to explain the potential antiarrhythmogenic effects of omega-3 fatty acids cannot be answered definitively at this time due to the limited number of studies for each outcome and the conflicting results obtained. Some trends were observed among the contractility and IPIM parameters, but these trends need further validation.