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Number 92

Effects of Omega-3 Fatty Acids on Arrhythmogenic Mechanisms in Animal and Isolated Organ/Cell Culture Studies

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Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-Based Practice Centers (EPCs), sponsors the development of evidence reports and technology assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. This report, *Effects of Omega-3 Fatty Acids on Arrhythmogenic Mechanisms in Animal and Isolated Organ/Cell Culture Studies*, was requested and funded by the Office of Dietary Supplements, National Institutes of Health. The reports and assessments provide organizations with comprehensive, science-based information on common, costly medical conditions and new health care technologies. The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments.

To bring the broadest range of experts into the development of evidence reports and health technology assessments, AHRQ encourages the EPCs to form partnerships and enter into collaborations with other medical and research organizations. The EPCs work with these partner organizations to ensure that the evidence reports and technology assessments they produce will become building blocks for health care quality improvement projects throughout the Nation. The reports undergo peer review prior to their release.

AHRQ expects that the EPC evidence reports and technology assessments will inform individual health plans, providers, and purchasers as well as the health care system as a whole by providing important information to help improve health care quality.

We welcome written comments on this evidence report. They may be sent to: Director, Center for Outcomes and Evidence, Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850.

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Structured Abstract

Context. Epidemiological studies and clinical trials have reported beneficial effects of fish or fish oil consumption on cardiovascular disease outcomes including sudden death and arrhythmia. The mechanisms of this reported benefit are, however, unclear.

Objectives. As one component of a series of reports on the impact of omega-3 fatty acids on cardiovascular disease, we also performed a systematic review of the literature on whole animal and isolated organ and cell culture studies to assess the effects of omega-3 fatty acids on arrhythmogenic mechanisms and outcomes.

Data Sources. We searched Medline, Embase, Biological Abstracts, and Commonwealth Agricultural Bureau databases for potentially relevant English language studies.

Study Selection. We screened over 1,807 abstracts and retrieved 295 full text articles. Eighty-six studies met our inclusion criteria and provided data to address the key questions in this report. We used comparative studies of whole animal, isolated organ and cells derived from omega-3 fatty acid-fed animals, and isolated organ and cell culture studies, in which the studies quantified the amount of omega-3 fatty acid in the intervention, to assess the effects of the interventions on arrhythmogenic mechanisms and outcomes.

Data Extraction. From each qualifying study, we extracted information about the study design, animal characteristics and model, the amount of omega-3 fatty acid used in the animal diet or in the experiments, the chemical agents used , the conditions under which the experiments were conducted, and outcomes. For whole animal studies, we extracted information about the randomization and blinding techniques to assess methodological quality.

Data Synthesis. Thirteen whole animal studies (rat models) were included in a meta-analysis that compared the anti-arrhythmic effects of ALA or fish oil to omega-6 oils. These meta-analysis results showed that fish oil supplementation showed a significant risk reduction in the number of deaths, ventricular tachycardia (VT), and ventricular fibrillation (VF). The combined risk ratio (RR) for deaths was 0.48 (95% CI: 0.24-0.95). With fish oil supplementation, for VT the RR was 0.49 (95%CI 0.29-0.83), and 0.68 (95%CI 0.50-0.91), for ischemia and reperfusion-induced arrhythmias, respectively. With fish oil supplementation, for VF, the RR was 0.21 (95%CI 0.07-0.63), and 0.44 (95%CI 0.25-0.79), for ischemia and reperfusion-induced arrhythmias, respectively. There was no significant effect for ALA oil supplementation, however.

There were twenty-one studies using isolated organs and cells from whole animals fed omega-3 fatty acids that examined the following parameters: contractile, basoelectromechanical, ion pumps and ion movements, ion currents, and ion channels. Although seven of these studies evaluated the effect of omega-3 fatty acid enriched diets on contractile parameters, they each compared different diets and used different experimental conditions.

Thirty-nine studies evaluated the effect of omega-3 fatty acids on isolated organ and cell cultures. Omega 3 fatty acids were applied either directly to the cell culture medium (free) or

incubated with the cells to allow incorporation into membrane phospholipids (bound). These studies examined parameters similar to the whole animal isolated organ and cell studies. Seven studies of arrhythmia reported that omega- 3 fatty acids (predominantly EPA and DHA but in one instance ALA) appeared to have a protective effect against spontaneous or induced arrhythmias in both rat and guinea pig models. Four of these studies, however, were from the same collaborative group. 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 inconsistent, with three showing a decrease in contractility and three showing no effect.

Conclusions. Fish oil supplementation (EPA and/or DHA) might have anti-arrhythmic effects when compared with omega-6, monounsaturated, or saturated fatty-acids in pre-fed fish oil in studies of various animal species. Fish oil supplements in rats showed significant protective effects for ischemia- and reperfusion- induced arrhythmias by reducing the incidence of ventricular tachycardia and fibrillation but no beneficial effects for ALA supplementation were found. The arrhythmic effects for infused omega-3 fatty-acid treatments are still unknown.

In studies using isolated organs and cells from animals fed omega-3 fatty acids and in studies using isolated organ and cell culture where fatty acids were directly applied to the culture medium, 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, despite some apparent trends. Due to numerous sub-parameters within each of the major electrogenesis areas (i.e. ion channels, ion currents, ion pumps and ion movement, contractility) studied , and a variety of experimental conditions, it is more difficult to draw a conclusion about the various parameters.

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Summary

Introduction

This evidence report is one of three prepared by the Tufts-New England Medical Center (Tufts-NEMC) Evidence-based Practice Center (EPC) concerning the health benefits of omega-3 fatty acids on cardiovascular diseases. These reports are among several that address topics related to omega-3 fatty acids that were requested and funded by the Office of Dietary Supplements, National Institutes of Health (NIH), through the EPC program at the Agency for Healthcare Research and Quality (AHRQ). Three EPCs—the Tufts-NEMC EPC, the Southern California/RAND EPC, and the University of Ottawa (UO) EPC—produced evidence reports. The aim of these reports is to summarize the current evidence of the health effects of omega-3 fatty acids on: cardiovascular diseases, cancer, child and maternal health, eye health, gastrointestinal/renal diseases, asthma, autoimmune diseases, immune-mediated diseases, transplantation, mental health, and neurological diseases and conditions. The focus of this report is on arrhythmogenic mechanisms in animal and isolated organ and cell culture studies.

Arrhythmias are thought to be the cause of "sudden death" in heart disease. Animal studies have suggested that omega-3 long-chain polyunsaturated fatty acids (LC PUFAs), such as eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), engage in multiple cytoprotective activities that may contribute to antiarrhythmic mechanisms.[1] In this report, we examine evidence that omega-3 fatty acids affect cell organelles—such as cardiac ion channels, pumps, or exchange mechanisms—that are involved in cardiac electrophysiology or electrogenesis.

The key questions addressed by this report are:

- What is the evidence from whole animal studies that omega-3 fatty acids affect arrhythmogenic outcomes (and intermediate outcomes)?
- What is the evidence from cell culture and tissue studies (including animal and human cardiac tissue) that omega-3 fatty acids directly affect cell organelles such as cardiac ion channels, pumps, or exchange mechanisms involved in electrogenesis?

In whole animal studies examined for this report, omega-3 fatty acids were fed to whole, intact animals as part of their diet or were infused intravenously. Outcomes examined by these studies include induced arrhythmia, ventricular ectopic beats, and ventricular and atrial fibrillation. In whole animal isolated organ and cell studies, omega-3 fatty acids were fed to whole, intact animals as part of their diet, and organs or cell tissues were subsequently excised from the animal to study outcomes such as arrhythmia, and myocyte contraction and beating rate. In "pure" isolated organ and cell studies, omega-3 fatty acids were applied directly to mammalian tissues or cultured cell lines or incorporated into the membrane of the mammalian tissues or cultured cell lines. Outcomes examined in these studies include induced arrhythmia, myocyte contraction and beating rate, and any other arrhythmogenic outcomes. In examining studies for this report, we focused on several potential arrhythmogenic mechanisms, including contractile parameters, basoelectromechanical parameters, ion pumps, ion channels, and membrane currents.

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Methods

Literature Search Strategy

This evidence report is based on a systematic review of the literature. Relevant studies were identified primarily through search strategies conducted in collaboration with the UO EPC. Preliminary searches were conducted at the Tufts-NEMC EPC using the OVID search engine on the MEDLINE® database. The final searches used five databases including:

- MEDLINE® from 1966 to week 2 of February 2003
- PreMEDLINE® from February 7, 2003
- EMBASE from 1980 to week 6 of 2003
- Biological Abstracts 1990 to December 2002
- Commonwealth Agricultural Bureau Health from 1973 to December 2002

A targeted search was conducted to retrieve articles that examined the effects of omega-3 fatty acids on cell organelles involved in electrophysiology. This search included in vivo as well as in vitro animal studies. MeSH® subject headings and text words were defined by reviewing key articles supplied by researchers and members of the technical expert panel. In addition, citation analyses of key articles were conducted using the Institute for Scientific Information's Web of Science—Science Citation Index® database. Publications that cited the key articles were scanned for appropriateness and for additional subject headings or text words. These additional headings and text words were then added to those used in the search strategy. The database searches were updated regularly, with the last update conducted on April 18, 2003.

Study Selection

Abstracts identified through the literature search were screened using eligibility criteria defined to include all English language primary experimental studies that evaluated the impact of omega-3 fatty acids on arrhythmia, intermediate mechanisms of arrhythmia, and electrogenesis. Reports published only as letters or abstracts were excluded. Articles associated with abstracts that passed these screens were retrieved and screened once more for eligibility. Studies were included if they examined the effect of omega-3 fatty acids on whole heart parameters (e.g., ventricular tachycardia, ventricular fibrillation), contractile parameters (e.g., heart rate, inotropic parameters), basoelectromechanical parameters (e.g., relative refractory period), ion pumps/movement (e.g., cytosolic calcium influx/efflux), ion currents (e.g., sodium currents), or ion channels (e.g., binding capacity).

Data Extraction

A standardized data extraction process was followed to ensure consistency across reviewers. Definitions for terms used in the extraction process were specified by consensus. As part of the training process, data was extracted from two of the same studies to compare interpretations. After this process, each study was partially screened to determine whether it met eligibility criteria and addressed relevant outcomes. Studies deemed eligible were then fully extracted by a single reviewer. Issues and discrepancies encountered during the extraction process were addressed at weekly meetings.

Analysis

We compiled detailed evidence tables describing study characteristics and results. Results were summarized with narrative descriptions of the evidence. Meta-analyses of whole animal studies were also conducted. For these analyses, we identified key measures and subgroups to construct random effects meta-analysis models using risk ratios.

Results

Literature Search Results

We identified 1,807 abstracts from the literature search. After screening the abstracts, we retrieved 274 articles. Of these, 183 were rejected after reviewing the full text articles. Reasons for rejection included: no omega-3 fatty acids, not specific to arrhythmia, no cardiac cells, fatty acid composition, or products only. Details for the reasons for rejection are summarized in the list of rejected articles included with the report. A total of 86 articles were accepted and reviewed.

Whole Animal Studies

Twenty-six whole animal studies were reviewed. Of these, 14 used rat models, seven used dog models, three used monkey models, one used a piglet model, and one used a rabbit model. Separate meta-analyses were performed for each of the outcomes studied. Findings related to the following subtopics are reviewed below.

- Arrhythmia deaths
- Ventricular tachycardia and ventricular premature beats
- Ventricular fibrillation and ventricular fibrillation threshold
- Arrhythmia severity
- Length of time in sinus rhythm

Arrhythmia deaths. The meta-analyses examining arrhythmia deaths included 12 comparisons from seven studies involving 150 rats fed omega-3 PUFAs and 152 rats fed omega-6 PUFAs. Five of the 12 comparisons compared the effects of alpha linolenic acid (ALA, 18:3 n-3) oils to omega-6 PUFA oils on deaths in ischemia-reperfusion-induced arrhythmias. The combined risk ratio of deaths for these five comparisons was 1.2 (95% confidence interval [CI]: 0.51-2.6; n=133). In contrast, the combined risk ratio of deaths for the other seven comparisons was 0.47 (95% CI: 0.24-0.95). The significantly reduced risk ratio of deaths in these seven comparisons, however, was due to a single study.[2] In a meta-analysis combining ALA and EPA plus DHA comparisons, the

overall risk ratio of deaths was 0.68 (95% CI: 0.40-1.2; n=169).

Ventricular tachycardia and ventricular premature beats. Ten comparisons were included in a meta-analysis of the risk ratio of ventricular tachycardia in ischemia-induced arrhythmias. Of these, four compared the effects of ALA oils to omega-6 PUFA oils on the incidence of ventricular tachycardia (VT). The combined risk ratio of deaths in these comparisons was 0.82 (95% CI: 0.65-1.0; n=248). Another 11 comparisons were combined to examine the effects of fish oils (EPA and DHA) on the incidence of VT in ischemia-induced arrhythmias. The combined risk ratio of deaths in these 11 comparisons was 0.49 (95% CI: 0.29-0.83; n=257). In a meta-analysis combining comparisons of ALA and EPA+DHA, the overall risk ratio of VT in ischemia-induced arrhythmias was 0.70 (95% CI: 0.53-0.92; n=76).

Eleven comparisons were included in a meta-analysis of reperfusion-induced arrhythmias. Of these, five compared the effects of ALA oils to omega-6 PUFA oils on the incidence of VT. The combined risk ratio of deaths in these five comparisons was 1.1 (95% CI: 0.73-1.6; n=125). The other six comparisons were combined to examine the effects of fish oils (EPA and DHA) on the incidence of VT in reperfusion-induced arrhythmias. The combined risk ratio of deaths in these comparisons was 0.68 (95% CI: 0.50-0.91; n=132). Combining comparisons of ALA and EPA plus DHA comparisons yielded an overall risk ratio of 0.85 (95% CI: 0.65-1.1; n=257) in reperfusion-induced arrhythmias.

Sixteen comparisons in seven studies compared the effects of omega-3 fatty acids and omega-6 fatty acids on ventricular premature beats (VPBs) in ischemia-induced and/or reperfusion-induced arrhythmias in rat models. A meta-analysis showed that rats fed fish oils had reduced numbers of VPBs in ischemia-induced and/or reperfusion-induced arrhythmias compared to rats fed omega-6 PUFA oils.

Several studies compared omega-3 fatty acids to saturated fatty acids. Three studies—one using a rabbit model, one using a piglet model, and one using a rat model—examined the numbers of VPBs in ischemia-reperfusion-induced arrhythmias. In the rat study, the numbers of VPBs during ischemia were significantly reduced among rats fed fish oil compared to those fed sheep-perirenal fat. In the piglet study, the incidence of VPBs was not different during ischemia, but during reperfusion significantly fewer VPBs were reported in piglets fed mackerel oil than in those fed lard fat. In the rabbit study, there were no significant differences in the incidence of VPBs between rabbits fed fish oil and those fed coconut oil during ischemia or reperfusion procedures.

Three infusion studies using dog models examined the effects of intravenously infused ALA on ischemia-induced or spontaneous arrhythmias. One study evaluated the incidence of VT in spontaneous arrhythmias among eight dogs. No events of VT or VPB were observed when infusing control buffer or ALA up to 10 mg/kg.

Ventricular fibrillation and ventricular fibrillation threshold. Eight comparisons were included in a meta analysis of the risk ratio of ventricular fibrillation (VF) in ischemia-induced arrhythmias. Three of the comparisons compared the effects of ALA oils and omega-6 PUFA oils on the incidence of VF in ischemia-induced arrhythmias. The combined risk ratio of deaths for these comparisons was 0.95 (95% CI: 0.56-1.6; n=76). The other five comparisons examined the effects of fish oils on the incidence of VF in ischemia-induced arrhythmias. The combined risk ratio of deaths for these comparisons was 0.21 (95% CI: 0.07-0.63; n=100). The meta-analysis combined comparisons of ALA and EPA plus DHA and showed that the overall random-effects risk ratio of VF in ischemia-induced arrhythmias was 0.69 (95% CI: 0.41-1.24; n=176).

Fourteen comparisons were included in a meta-analysis of the incidence of VF in reperfusion-induced arrhythmias. Of these, six compared the effects of ALA oils to omega-6 PUFA oils. The combined risk ratio of deaths in these six comparisons was 0.84 (95% CI: 0.52-1.3; n=144) with heterogeneity present. The other eight comparisons examined the effects of fish oils on the incidence of VF in reperfusion-induced arrhythmias. The combined risk ratio of death for these comparisons was 0.44 (95% CI: 0.25-0.79; n=168). In the meta-analysis combining ALA and EPA plus DHA comparisons, the overall random-effect risk ratio of VT in reperfusion-induced arrhythmias was 0.85 (95% CI: 0.65-1.1; n=312).

Three studies examined the incidence of VF and ventricular fibrillation threshold (VFT) in induced arrhythmia. These studies compared monkeys fed fish oils to controls fed sunflower seed oil (an omega-6 PUFA). The studies found no difference in the proportion of monkeys with inducible VF in normal conditions. Under ischemic conditions, two of the three studies found no difference in the proportion of monkeys with induced VF. The third study reported that no VF was induced in the monkeys fed fish oil, but 13 percent of the monkeys fed sunflower seed oil had induced VF. Among monkeys receiving isoproterenol, VF was induced in 30 percent to 50 percent of the monkeys fed fish oils compared to 77 percent to 100 percent of the monkeys fed sunflower seed oil. VFTs were measured only among VF-inducible monkeys. In two studies comparing monkeys fed fish oil to those fed sunflower seed oils, there were no changes in VFTs in all conditions.

Arrhythmia severity. Eight studies representing 18 comparisons used rat models to evaluate arrhythmia scores of ischemia-induced and/or reperfusion-induced arrhythmias. More severe arrhythmias were associated with higher scores. No consistent results were found in studies comparing rats fed ALA oils (soybean, linseed, or canola oils) to those fed omega-6 PUFA oils. However, studies comparing rats fed fish oils to rats fed omega-6 PUFA oils found that most rats fed fish oils had less severe ischemia-induced and/or reperfusion-induced arrhythmias.

Length of time in sinus rhythm. Three studies representing seven comparisons used rat models to evaluate the length of time in sinus rhythm (TSR) in ischemia-induced and/or reperfusion-induced arrhythmias. One study compared rats fed linseed oil (which is rich in ALA) to those fed corn oil and found no significant difference in TSR in ischemia-induced arrhythmias. However, the same study found that TSR in ischemia-induced arrhythmias was significantly increased in rats fed fish oil compared to rats fed corn oil. The other two studies that compared rats fed fish oils to rats fed omega-6 PUFA oils found no significant difference in TSR in ischemia-induced and/or reperfusion-induced arrhythmias. Two studies using rat models directly compared EPA+DHA to ALA. Both reported a non-significant reduction in the incidence of VT and VF in ischemia-induced or reperfusion-induced arrhythmias in rats fed fish oils compared to those fed soybean or linseed oils. Five studies compared omega-3 fatty acids to saturated fatty acids. Deaths in ischemia-reperfusion-induced arrhythmias were observed in two of these studies.

Whole-Animal Isolated Organ and Cell Studies

Twenty-one studies used isolated organs and cells from whole animals to examine contractile parameters, basoelectromechanical parameters, ion pumps and ion movements, ion currents, and ion channels. Findings related to several of these parameters are discussed below.

Contractile parameters. Three studies showed that fish oil or EPA+DHA supplementation did not change heart rate. One study showed that, in the presence of an arrhythmogenic agent, fish oil significantly decreased heart rate compared to safflower oil. One study examined the effect of cod liver oil supplementation on heart rate and showed a significant decrease under some, but not all, conditions.

Basoelectromechanical parameters. One study using a rat model showed that supplementing a high fat diet with fish oil significantly reduced the ventricular effective refractory period. Another study using a rabbit model showed no effect of dietary fish oil compared to safflower oil on the ventricular effective refractory period, absolute refractory period, relative refractory period, or epicardial or endocardial monophasic action potential.

Ion pump. Several studies examined ion pump activity using mouse models. One compared a diet enriched with EPA ester or DHA to a diet containing safflower oil and found no difference in sarcoplasmic reticulum (SR) calcium-magnesium adenosine triphosphatase (ATPase) activity. Another study compared fish oil to corn oil and showed a significant decrease in SR calcium-magnesium ATPase activity. A third study showed that, compared to standard chow diet, supplementation with graded doses of DHA ester did not affect calcium-magnesium ATPase activity in the SR, but significantly increased calcium-magnesium ATPase in the cardiac myocyte at low doses. At a higher dose, however, there was no change. One study using a rat model measured SR calcium-magnesium ATPase, calcium ATPase, and magnesium ATPase using graded doses of adenosine triphosphate (ATP) and ionomycin. This study found significant decreases with fish oil supplementation compared to a corn-oil based diet. A study using a canine model reported significant increases in cardiac calcium-magnesium ATPase with EPA ester supplementation. Three studies (two rat and one canine model) all reported no change in sodium-potassium ATPase activity with an omega-3 fatty acid diet regardless of dosage or agent used.

Three studies using rat models examined the effect of fish oil supplementation on cytosolic calcium content. Each of the studies reported there was no difference under ambient conditions between fish oil supplementation and an omega-6 fatty acid diet or a saturated fatty acid diet.

Two studies showed a significant decrease in SR calcium content with omega-3 fatty acids and fish oil. One, using a mouse model, compared an omega-3 fatty acid to a safflower oil control while the other used a rat model and compared fish oil to corn oil. Two studies (one using a mouse model and the other using a rat model) compared fish oil to corn oil and reported significant decreases in SR calcium uptake with fish oil.

Isolated Organ and Cell Culture Studies

We identified 39 studies that examined the effects of omega-3 fatty acids on isolated organs and cell cultures. Key findings related to the following subtopics are summarized below.

- Arrhythmogenic and contractile parameters
- Basoelectromechanical parameters
- Ion pumps and ion movements
- Ion currents
- Ion channels

Arrhythmogenic and contractile parameters. Four studies using rats demonstrated that free-EPA or DHA significantly prevented or terminated the proportion of arrhythmias induced by various agents. Another study demonstrated that free omega-3 fatty acids were effective in terminating induction of arrhythmias while bound omega-3 fatty acids were not. Another study using a rat model showed that bound-DHA significantly decreased the proportion of arrhythmias induced by nor-adrenaline and timolol. A study using a guinea pig model showed that free-EPA (sodium salt) at a low dosage did not have an effect on antigen-induced arrhythmia but produced a significant decrease in the proportion of induced arrhythmias at a high dosage.

A number of contractility studies compared the effect of free ALA, EPA, or DHA, alone or in combination, to a control in the absence of any agent. Three of the studies showed no effect on contractility, while three showed a decrease. Among studies that used an agent to examine contractility, all demonstrated a decrease in contractility, or a protective effect of the omega-3 fatty acids in blocking the negative response induced by the agents. One study also showed that DHA blocked the

inhibitory effect of nitrendipine on myocyte contraction, but not the inhibitory effect of verapamil and diltiazem. Three studies examined the effect of methylated (m.e.) or ethylated (e.e.) free-EPA or DHA on contractility. Two used rat models and showed that free-EPA e.e. in the absence of an agent, or free-DHA m.e. in the presence of isoproteronol, had no effect on contractility. The third study examined a different contractile parameter.

Two studies examined the effect of omega-3 fatty acids on twitch size, and both used rat and guinea pig models.[3,4] The two guinea pig models observed a decrease in twitch size with free-EPA and/or free-DHA. One of the rat models observed an increase in twitch size with EPA or DHA at concentrations between 1-7.5 µm, and a decrease in twitch size with concentrations >10 µM.[3] The other rat model observed a significant decrease in twitch size with 5 µM of EPA.[4]

Three studies examined the effect of omega-3 fatty acids on inotropic parameters. One study used a rat model and reported that neither free-EPA nor DHA had an effect on amplitude of contraction. Another study used bound-EPA with a rat model and showed no change in amplitude of contraction. However, the same study found that amplitude increased significantly with ouabain. The third study examined a different inotropic parameter.

A number of studies examined contractility parameters. Two studies compared bound omega-3 fatty acids to bound omega-6 fatty acids under ambient, hypoxic, and reoxygenated conditions and showed no effect on the contractility parameters that were investigated. Four studies compared bound-EPA to bound-DHA and found no difference in their effects on contraction duration at 20 percent relaxation (CD_{20}), contraction duration at 80 percent relaxation (CD_{80}), relaxation time (–Cmax), and cell shortening velocity (+ Cmax) regardless of the agents used to induce arrhythmia. One study compared bound-ALA+ EPA to omega-6 fatty acids and reported no difference in CD_{80} and –Cmax, but found a significant increase in +Cmax.

Basoelectromechanical parameters. One study reported an increase in the action potential[5] with free-EPA compared to a control, while another study (also using free-EPA) reported a significant decrease in both the action potential and the frequency of the action potential.[6] In the presence of three different agents (sodium and timolol, isoproteronol, and ouabain), bound-DHA significantly decreased the action potential compared to control. No change was observed in the absence of an agent.[7] Two studies compared bound synthesized medium for omega-3 group (SM3) to bound synthesized medium for omega-6 group (SM6) and reported no change in the action potential under ambient, hypoxic, and reoxygenated conditions. Two studies showed that 5-10 µM of free-EPA and/or DHA did not affect the action potential amplitude (APA) compared to control, but concentrations >10-50 µM yielded a significant decrease in APA. One study compared the effect of bound-DHA relative to control and reported a significant increase in action potential amplitude using EPA. Two studies examined the effects of omega-3 fatty acid combinations (SM3) versus omega-6 fatty acids (SM6) under varying conditions. Both showed no change in APA under ambient conditions and a significant decrease in APA under hypoxic conditions. However, under the reoxygenation condition, the results differed: one study reported no change and the other reported a significant increase in APA. Two studies compared the effect of bound-EPA to bound-DHA and found that EPA significantly increased APA compared to DHA.

Four studies using rat models examined the effect of omega-3 fatty acids on the action potential duration at 40 percent polarization. One of these studies reported an increase in this parameter in the presence of both free-EPA and free-DHA compared to control.[5] Two of the studies compared bound-SM3 to bound-SM6 under three experimental conditions. One reported no change under all three conditions, while the other reported a significant decrease in action potential duration at 40 percent polarization under hypoxic conditions but no change under ambient or reoxygenation conditions. One study compared bound-EPA to bound-DHA and did not find a differential effect on this basal electromechanical parameter.

Five studies using rat models and one study using both a rat and guinea pig model examined the effect of omega-3 fatty acids on the action potential duration at 80 percent polarization (APD_{80}). One of the studies compared free-EPA (10 µM) to control and reported a significant decrease in APD_{80}.[6] Similarly, another study reported a dose-dependent decrease in APD_{80} with EPA concentrations >10 µM, but an increase with EPA concentrations between 1 and 7.5 µM.[3] The same authors also used a guinea pig model and reported that EPA was effective in decreasing APD_{80} at concentrations between 1 and 20 µM. One study compared bound-SM3 to SM6 and reported no change in APD_{80} under hypoxic, ambient, or reoxygenation conditions, while another study reported a significant decrease in action potential duration at 40 percent (APD_{40}) polarization under hypoxic conditions, but no change under ambient or reoxygenation conditions. Two studies compared bound-EPA to bound-DHA and observed no effect on action potential.

Several studies examined the effects of omega-3 fatty acids on the maximum rate of depolarization (V_{max}). One study demonstrated a increase in V_{max} with either free-EPA or free-DHA compared to control. Two studies compared bound-SM3 to bound-SM6 under varying experimental conditions. One reported no change in V_{max} under any of three conditions, while the other reported a significant increase in Vmax under ambient conditions, but no change under either hypoxic or reoxygenated conditions. Two studies compared bound-EPA to bound-DHA and found no difference in V_{max}.

Several studies examined overshoot potential (OS). One study compared bound-SM3 to bound-SM6 and reported no effect on OS. Another study also compared bound-SM3 to

SM6, but under varying experimental conditions, and found that bound-SM3 did not affect OS differently from bound-SM6 under ambient conditions. However, bound-SM3 significantly decreased OS under hypoxic conditions and significantly increased OS during reoxygenation. Two studies compared bound-EPA to bound-DHA and reported that bound-EPA significantly increased OS compared to bound-DHA.

Ion pumps and ion movements. Three studies examined the effect of omega-3 fatty acids on cytosolic calcium influx. One study using a rat model reported that free-EPA decreased cytosolic calcium influx.[8] The second study found that free-DHA blocked the effect of nitrendipine and Bay8644 (BAY) on cytosolic calcium influx.[9] Another study that used a rat model examined the effect of bound-EPA or bound-DHA in the presence of several agents. This study found that DHA blocked the ouabain-induced increase in cytosolic calcium influx. It also showed that both EPA and DHA blocked the nitrendipine-induced decrease, ouabain+nitrendipine-induced decrease, BAY+nitrendipine-induced decrease, and the BAY-induced increase in cytosolic calcium influx.

Seven studies examined the effect of omega-3 fatty acids on cytosolic calcium content. One study directly compared the effects of acute and chronic exposure to free-DHA. This study showed that both acute and chronic exposure were effective in lessening the magnitude of increase in cytosolic calcium content induced by an agent (potassium chloride) or an anoxic condition.[10] While two studies showed that free-EPA decreased cytosolic calcium content, the other four studies showed that neither free- nor bound-EPA or DHA had an effect. In the presence of various agents, free- or bound-EPA and DHA blocked the alterations in cytosolic calcium induced by the agents.

Four studies examined the effect of omega-3 fatty acids on sodium-calcium and sodium-hydrogen exchangers. Two used a canine model and reported that free-ALA increased sodium-calcium exchange.

Ion currents. Twelve studies examined the effect of free omega-3 fatty acids on ion currents in isolated organs or cells. One study using a rat model demonstrated a significant shift in the voltage dependence of activation to more positive potentials with ALA, EPA, or DHA. The same study also demonstrated a significant shift in the inactivation of the sodium current to more negative potentials with ALA, EPA, or DHA.[11] A study using both rat and guinea pig models found a dose–dependent decrease in peak amplitude of the sodium current with both EPA and DHA.[3] In another study using a rat model, a significant time, dose, and voltage-dependent decrease of the sodium current was observed using ALA, EPA, or DHA. However, there was no change in the current-voltage relationship and the activation or inactivation parameters of the sodium current.

Three studies used rat models to examine transient potassium outward current (I_{to}). One of the studies showed that both EPA and DHA decreased I_{to} amplitude and the time constant of I_{to} inactivation and increased the I_{to} delay.[5] The presence of indomethacin did not modify these effects. In the second study, there was a dose-dependent decrease in I_{to},[3] and in the third study, EPA significantly decreased the frequency and significantly increased the amplitude of I_{to}[8]. A study using ferrets showed that ALA, EPA, or DHA significantly decreased I_{to} amplitude.[12]

Six studies examined the effects of free omega-3 fatty acids on the voltage-dependent L-type calcium current ($I_{Ca.L}$). One study using a rat and guinea pig model found that both EPA and DHA caused a dose-dependent decrease in $I_{Ca.Lma}$.[3] In another rat study, both EPA and DHA decreased the amplitude of $I_{Ca.L}$.[13] A study examining the effect of various agents on $I_{Ca.L}$ showed that DHA increased the current amplitude in the presence of nitrendipine. DHA also blocked the BAY K8644-induced increase in $I_{Ca.L}$ amplitude, but did not change the amplitude in the presence of isoproteronol.[9] Another study using a rat model showed that significant time, dose, voltage-dependent decreases in $I_{Ca.L}$, and a negative shift in the $I_{Ca.L}$ inactivation curve occurred in the presence of ALA, EPA, or DHA. In a study using a guinea pig model and methylated DHA, a significant increase in $I_{Ca.L}$ was observed.[14] Another guinea pig model showed that EPA produced a significant decrease.[4]

Four studies examined the effect of free omega-3 fatty acids on inward rectifier potassium current (I_{KI}). A study using a mouse model showed no effect of DHA on I_{KI}, and a study using a rat model showed no effect of either EPA or DHA.[5] Another study using EPA with rat and guinea pig models showed a decrease in I_{KI},[3] while a study using a ferret model showed no change using either ALA, EPA, or DHA.[12]

Ion channels. Three studies examined ion channels in isolated organs or cells. Because each study examined different parameters, the conclusions that can be inferred from these studies are limited.

Discussion

In conclusion, based on the meta-analyses of the incidences of total deaths and of ventricular tachycardia and ventricular fibrillation in ischemia- and/or reperfusion-induced arrhythmias, fish oil supplementation has anti-arrhythmic effects in the rat model when compared to omega-6-fatty acid supplementation. 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 on ischemia- and/or reperfusion-induced arrhythmias in the rat model were found for ALA supplementation compared to omega-6-fatty acid supplementation. Results were consistent in the two studies that directly compared 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 compared to rats fed soybean or linseed oils.

In monkey models, fish oil supplementations were found to prevent deaths in ischemia- and isoproterenol-induced arrhythmias in one study. In addition, three 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. Five studies showed consistent protective effects on ischemia- and/or reperfusion-induced arrhythmias in rats, rabbits, or pigs fed fish oils compared to rats fed saturated fatty acids, although again the results were not statistically significant for most comparisons.

In comparison to omega-6, monounsaturated, or 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 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. The mechanisms of the observed anti-arrhythmic effects of albumin-bound ALA, EPA, and 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 are unknown.

In studies using whole isolated organ and cell culture studies 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 ion pumps and ion movement parameters, but these trends need further validation.

Limitations and Recommendations

Synthesizing data regarding the effects of omega-3 fatty acids on arrhythmogenic mechanisms was complicated by a number of issues. Several of these are discussed below and recommendations for future studies are highlighted.

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 a study and the reliability of study results. Many of these factors were not observed in the 26 whole-animal studies reviewed. For example, only three 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 prior papers was common. Contemporary controls were used in all but monkey and infusion studies. Exclusion criteria were rarely used.

In addition, while 26 whole-animal studies were identified, approximately 70 percent of studies included in the meta-analyses are from the same group of collaborating researchers, which to some degree accounts for the standardization of arrhythmic outcome measures. The results reported from one 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.

With respect to study design, standardized measures are needed, especially for isolated organ and cell culture studies. Research would be more interpretable if core sets of standardized measures that produce the highest information yield were agreed upon. We grouped outcomes reported in the various studies into five major categories to aid in the summary of results. However, we found wide variation in reports of the same outcome due to different experimental methodologies.

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. It appears, however, that the results are not always applicable across species, all cardiac cell types used (atrial, ventricular, etc.), and all development stages (neonatal, adult). It would, therefore, be useful 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.

We found that the concentrations of omega-3 fatty acids used in the isolated organ and cell culture studies were markedly different (1 μM to 214 μM). The results obtained at concentrations greater than 20 μM are questionable due to non-specific effects such as detergent effects on ion channels. 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 NIH or other suppliers to all researchers with a valid protocol. Additionally, a consensus needs to be reached on dosage.

While most studies reported results compared to a control, it might be more relevant to use an omega-6 fatty acid or a

monounsaturated fatty acid as the comparison group. Additionally, only three studies evaluated the effect of one omega-3 fatty acid compared to another omega-3 fatty acid. This area needs further research.

Classifying studies by experimental condition and agent used is problematic. It might be appropriate to convene an expert panel to evaluate and standardize available methodologies (ischemic models versus arrhythmogenic models) that are more relevant to the human situation so that the results are comparable across studies and are more applicable or generalizable to humans.

Availability of the Full Report

The full evidence report from which this summary was taken was prepared for the Agency for Healthcare Research and Quality (AHRQ) by the Tufts-New England Medical Center Evidence-based Practice Center, Boston, MA, under Contract No. 290-02-0022. It is expected to be available in March 2004. At that time, printed copies may be obtained free of charge from the AHRQ Publications Clearinghouse by calling 800-358-9295. Requesters should ask for Evidence Report/Technology Assessment No. 92, *Effects of Omega-3 Fatty Acids on Arrhythmogenic Mechanisms in Animal and Isolated Organ/Cell Culture Studies.* In addition, Internet users will be able to access the report and this summary online through AHRQ's Web site at www.ahrq.gov.

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Chapter 1. Introduction

This evidence report is one of three reports prepared by the Tufts-New England Medical Center (Tufts-NEMC) Evidence-based Practice Center (EPC) concerning the health benefits of omega-3 fatty acids on cardiovascular diseases. These reports are among several that address topics related to omega-3 fatty acids, and that were requested and funded by the Office of Dietary Supplements, National Institutes of Health (NIH) through the EPC program at the Agency for Healthcare Research and Quality (AHRQ). Three EPCs — the Tufts-NEMC EPC, the Southern California-RAND EPC, and the University of Ottawa EPC — each produced evidence reports. To ensure consistency of approach, the three EPCs collaborated on selected methodological elements, including literature search strategies, rating of evidence, and data table design.

The aim of these three reports is to summarize the current evidence on the health effects of omega-3 fatty acids on the following: cardiovascular diseases, cancer, child and maternal health, eye health, gastrointestinal/renal diseases, asthma, autoimmune diseases, immune-mediated diseases, transplantation, mental health, and neurological diseases and conditions. In addition to informing the research community and the public on the effects of omega-3 fatty acids on various health conditions, it is anticipated that the findings of the reports will also be used to help define the agenda for future research.

The focus of this report is on the effect of omega-3 fatty acids on cardiac electrogenesis and arrhythmias. The other two reports focus on the effects of omega-3 fatty acids on cardiovascular disease and effects of omega-3 fatty acids on cardiovascular disease risk factors. In this chapter, we review the metabolism, physiological functions, and sources of omega-3 fatty acids. In addition, we examine some basic aspects of cardiac electrophysiology or electrogenesis and discuss the analytic framework for this report. Subsequent chapters describe the methods used to identify and review studies related to omega-3 fatty acids and cardiac electrogenesis, findings related to the effects of omega-3 fatty acids on cardiac electrogenesis and arrhythmias, and recommendations for future research in this area.

Metabolism and Biological Effects of Essential Fatty Acids

Dietary fat is an important source of energy for biological activities in human beings. Dietary fat encompasses saturated fatty acids, which are usually solid at room temperature, and unsaturated fatty acids, which are liquid at room temperature. Unsaturated fatty acids can be further divided into monounsaturated and polyunsaturated fatty acids. Polyunsaturated fatty acids (PUFAs) can be classified on the basis of their chemical structure into two groups: omega-3 (n-3) fatty acids and omega-6 (n-6) fatty acids. The *omega-3* or *n-3* notation means that the first double bond from the methyl end of the molecule is in the third. The same principle applies to the *omega-6* or *n-6* notation. Despite their differences in structure, all fats contain the same amount of energy (9 kcal/g or 37 kJ/g).

Of all fats found in food, 2 — alpha-linolenic acid (chemical abbreviation: ALA, 18:3 n-3) and linoleic acid (LA, 18:2 n-6) — cannot be synthesized in the human body, yet are necessary for proper physiological functioning. These 2 fats are called essential fatty acids. The essential fatty acids can be converted in the liver to long-chain polyunsaturated fatty acids (LC PUFAs), which

have a higher number of carbon atoms and double bonds. These LC PUFAs retain the omega type (n-3 or n-6) of the parent essential fatty acids.

ALA and LA comprise the bulk of the total PUFAs consumed in a typical North American diet. Typically, LA comprises 89% of the total PUFAs consumed, while ALA comprises 9%. Smaller amounts of other PUFAs make up the remainder [1]. Both ALA and LA are present in a variety of foods. For example, LA is present in high concentrations in many commonly used oils, including safflower, sunflower, soy, and corn oil. ALA, which is consumed in smaller quantities, is present in leafy green vegetables and in some commonly used oils, including canola and soybean oil. Some novelty oils, such as flaxseed oil, contain relatively high concentrations of ALA, but these oils are not commonly found in the food supply.

The Institute of Medicine suggests that, for adults 19 and older, an adequate intake (AI) of ALA is 1.1-1.6 g/day, while an adequate daily intake of LA is 11-17 g/day [2]. Recommendations regarding AI differ by age and gender groups, and for special conditions such as pregnancy and lactation.

As shown in Figure 1.1, EPA and DHA can act as competitors for the same metabolic pathways as AA. In human studies, the analyses of fatty-acid compositions in both blood phospholipids and adipose tissue showed similar competitive relationship between omega-3 LC PUFAs and AA. General scientific agreement supports an increased consumption of omega-3 fatty acids and reduced intake of omega-6 fatty acids to promote good health. However, for omega-3 fatty acid intakes, the specific quantitative recommendations vary widely among countries not only in terms of different units — ratio, grams, total energy intake — but also in quantity [3]. Furthermore, there remain numerous questions relating to the inherent complexities about omega-3 and omega-6 fatty acid metabolism, in particular regarding the inter-relationships between the 2 fatty acids. For example, it remains unclear to what extend ALA is converted to EPA and DHA in humans, and to what extend high intake of omega-6 fatty acids compromises any benefits of omega-3 fatty acid consumption. Without resolution of these 2 foundational questions, it remains difficult to study the importance of omega-6 to omega-3 fatty acid ratio.

Metabolic Pathways of Omega-3 and Omega-6 Fatty Acids

Omega-3 and omega-6 fatty acids share the same pools of enzymes and go through the same oxidation pathways while being metabolized (Figure 1.1). Once ingested, ALA and LA can be elongated and desaturated into LC PUFAs. LA is converted into gamma-linolenic acid (GLA, 18:3 n-6), an omega-6 fatty acid that is a positional isomer of ALA. GLA, in turn, can be converted to the long-chain omega-6 fatty acid, arachidonic acid (AA, 20:4 n-6). ALA can be converted, to a lesser extent, to the long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3). However, the conversion from parent fatty acids into LC PUFAs occurs slowly in humans, and conversion rates are not well understood. Because of the slow rate of conversion and the importance of LC PUFAs to many physiological processes, humans must augment their level of LC PUFAs by consuming foods that are rich in these important compounds. Meat is the primary food source of AA, while fish is the primary food source of EPA.

The specific biological functions of fatty acids depend on the number and position of double bonds and the length of the acyl chain. Both EPA and AA are 20-carbon fatty acids and are precursors for the formation of prostaglandins, thromboxane, and leukotrienes — hormone-like

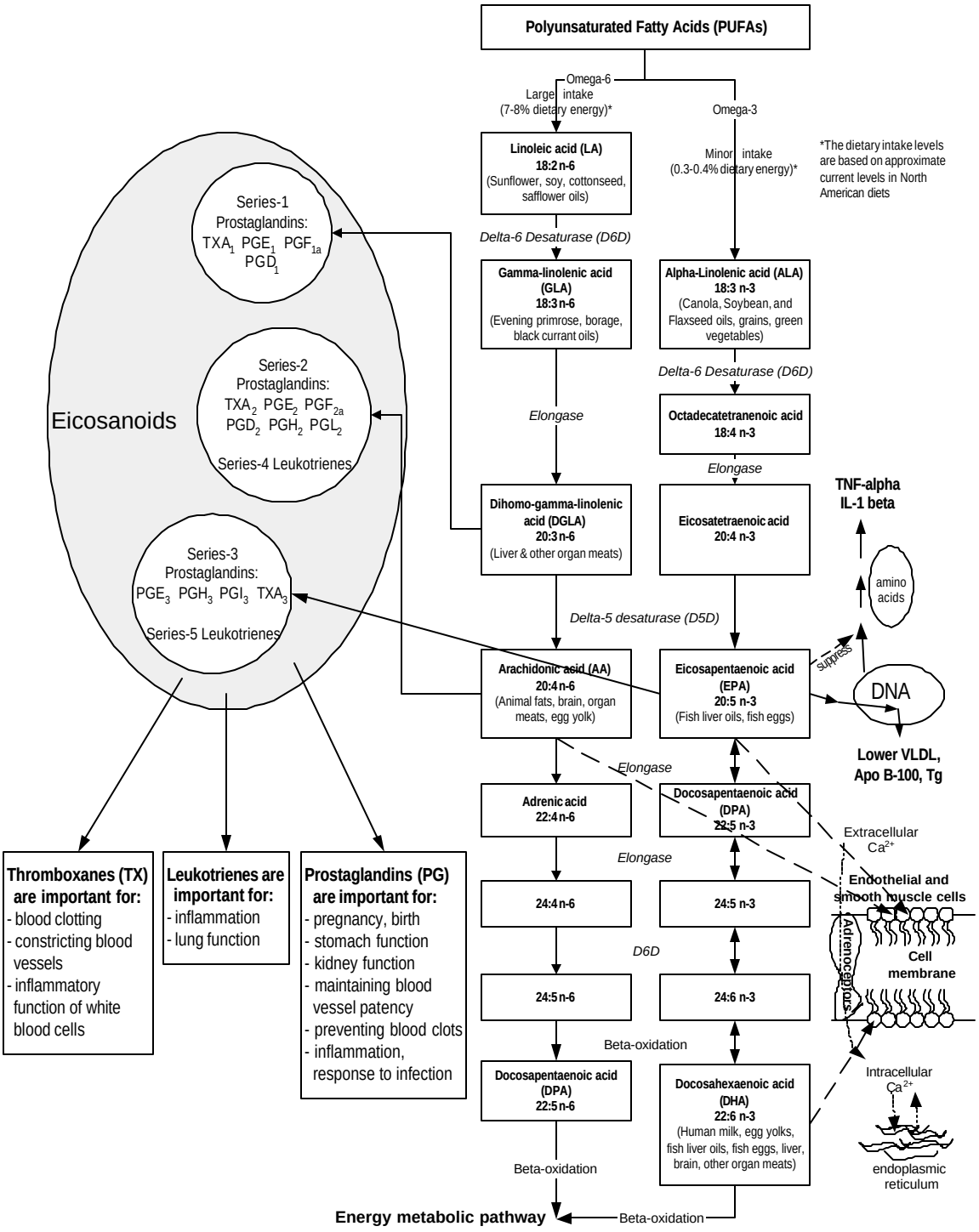
agents that are members of a larger family of substances called eicosanoids. Eicosanoids are localized tissue hormones that seem to be one of the fundamental regulatory classes of molecules in most higher forms of life. They do not travel in the blood, but are created in the cells to regulate a large number of processes, including the movement of calcium and other substances into and out of cells, dilation and contraction of muscles, inhibition and promotion of clotting, regulation of secretions including digestive juices and hormones, and control of fertility, cell division, and growth [4].

As shown in Figure 1.1, the long-chain omega-6 fatty acid, AA, is the precursor of a group of eicosanoids including series-2 prostaglandins and series-4 leukotrienes. The omega-3 fatty acid, EPA, is the precursor to a group of eicosanoids including series-3 prostaglandins and series-5 leukotrienes. The series-2 prostaglandins and series-4 leukotrienes derived from AA are involved in intense actions (such as accelerating platelet aggregation and enhancing vasoconstriction and the synthesis of inflammatory mediators) in response to physiological stressors. The series-3 prostaglandins and series-5 leukotrienes that are derived from EPA are less physiologically potent than those derived from AA. More specifically, the series-3 prostaglandins are formed at a slower rate and work to attenuate excessive series-2 prostaglandins. Thus, adequate production of the series-3 prostaglandins, which are derived from the omega-3 fatty acid, EPA, may protect against heart attack and stroke as well as certain inflammatory diseases like arthritis, lupus, and asthma [4]. In addition, animal studies, have demonstrated that omega-3 LC PUFAs, such as EPA and DHA, engage in multiple cytoprotective activities that may contribute to antiarrhythmic mechanisms[5]. Arrhythmias are a common cause of "sudden death" in heart disease.

In addition to affecting eicosanoid production as described above, EPA also affects lipoprotein metabolism and decreases the production of other compounds — including cytokines, interleukin 1ß (IL-1ß), and tumor necrosis factor a (TNF-a) — that have pro-inflammatory effects. These compounds exert pro-inflammatory cellular actions that include stimulating the production of collagenases and increasing the expression of adhesion molecules necessary for leukocyte extravasation [6]. The mechanism responsible for the suppression of cytokine production by omega-3 LC PUFAs remains unknown, although suppression of eicosanoid production by omega-3 fatty acids may be involved. EPA can also be converted into the longer chain omega-3 form of docosapentaenoic acid (DPA, 22:5 n-3), and then further elongated and oxygenated into DHA. EPA and DHA are frequently referred to as very long chain omega-3 fatty acids. DHA, which is thought to be important for brain development and functioning, is present in significant amounts in a variety of food products, including fish, fish liver oils, fish eggs, and organ meats. Similarly, AA can convert into an omega-6 form of DPA. Studies have reported that omega-3 fatty acids decrease triglycerides (Tg) and very low density lipoprotein (VLDL) in hypertriglyceridemic subjects, with a concomitant increase in high density lipoprotein (HDL). However, they appear to increase or have no effect on low density lipoprotein (LDL). Omega-3 fatty acids apparently lower Tg by inhibiting VLDL and apolipoprotein B-100 synthesis and decreasing post-prandial lipemia [7]. Omega-3 fatty acids, in conjunction with transcription factors (small proteins that bind to the regulatory domains of genes), target the genes governing cellular Tg production and those activating oxidation of excess fatty acids in the liver. Inhibition of fatty acid synthesis and increased fatty acid catabolism reduce the amount of substrate available for Tg production [8]

As noted earlier, omega-6 fatty acids are consumed in larger quantities (>10 times) than omega-3 fatty acids. Maintaining a sufficient intake of omega-3 fatty acids is particularly important since many of the body's physiologic properties depend upon their availability and metabolism.

Figure 1.1. Classical omega-3 and omega-6 fatty acid synthesis pathways and the role of omega-3 fatty acid in regulating health/disease markers.



Overview of the Electrophysiology of the Heart

In this report, we examine evidence that omega-3 fatty acids affect cell organelles — such as cardiac ion channels, pumps, or exchange mechanisms — that are involved in cardiac electrophysiology or electrogenesis. This section of the report reviews some basic aspects of electrogenesis and omega-3 fatty acids., and discusses the analytic framework that guided our systematic review of the literature. Two accompanying reports --- *Effects of Omega-3 Fatty Acids on Cardiovascular Disease Risk Factors* and *Effects of Omega-3 Fatty Acids on Cardiovascular Disease* review evidence from clinical studies focused on the relationship between omega-3 fatty acids and outcomes in humans including sudden death.

Cardiac Electrophysiology

The heart's beating rate is controlled by specialized, spontaneously firing pacemaker cells in the sino-atrial node (a bundle of specialized cardiac muscle cells in the right atrium of the heart) and by sympathetic and parasympathetic nerve fibers that influence the ion balance in heart muscle cells. The pacemaker cells initiate an electrical impulse that produces a change in the voltage of heart cell membranes. This change in voltage, also called an action potential, is generated by the relative concentration of different types of ions across the cell membrane, and moves from one heart muscle cell (or myocyte) to another [9].

Calcium, potassium, and sodium ions are central to the generation of action potentials. These ions, in the form of currents, move across cell membranes through pathways called channels. The speed at which ions traverse these channels varies due to channel characteristics. Some channels open or close as a function of membrane potential, while others respond to neurotransmitters or other molecules. Sodium and calcium ions also use an energy-dependent pumping process to cross the membrane. [9].

These electrophysiological processes interact with structural components of cardiac myocytes to cause synchronized contraction and relaxation of the heart muscle. The sarcoplasmic reticulum (SR) — a system of membranes in cardiac muscle cells— stores calcium ions during the diastolic, or relaxation, phase of the contraction cycle. Infoldings of the cell membrane (or sarcolemma) called T-tubules transmit the action potential along the membrane far into the cell. The resulting excitation-contraction coupling process increases the concentration of intracellular free calcium ions during depolarization across the cell membrane and T-tubules. The calcium ions facilitate muscle contraction by interacting with other cellular components. The exchangers and pumps that support the contractile process rely on the presence of adenosine triphosphate (ATP) and are affected by the concentration gradients of sodium, potassium, and calcium ions. The strength of cardiac muscle contraction, or myocardial contractility, can be increased by norepinephrine, which is secreted by sympathetic nerves and mediated by ß-adrenergic receptors and calcium channels. Myocardial muscle relaxation occurs when the calcium is returned to the sarcoplasmic reticulum or is pumped out of the cell by sodium-calcium exchangers and calcium adenosine triphosphatase (ATPase) pumps [9].

Arrhythmia

Cardiac arrhythmias, or disorders of the heart's rhythm, are a serious cause of morbidity and mortality. Serious arrhythmias can cause sudden death (abrupt loss of heart function or cardiac arrest) — a leading cause of death in industrialized societies. According to the Heart and Stroke Statistical Update for 2003 [10] arrhythmias were a direct cause of 37,646 deaths in the United States and were an underlying or contributing cause of another 491,000 deaths. In addition to contributing to sudden death, serious arrhythmias can compromise the normal flow of blood through the coronary arteries, resulting in impaired oxygenation of the heart muscle (myocardial ischemia) or death of cardiac muscle tissue (myocardial infarction or heart attack). Arrhythmias can also lead to other cardiovascular conditions, such as stroke, congestive heart failure, and peripheral embolism.

There are many potential causes of arrhythmias, including disruption of ion channels or pumps, reduction in blood flow to the heart muscle (ischemia), and alteration of the eicosanoid system and adrenoceptors (membrane proteins whose function in the heart is to transmit the neuroendocrine message sent by catecholamines like adrenaline and its derivatives). Changes in these systems result in electrical abnormalities in the heart leading to disturbances in the heart rhythm such as tachycardia, bradycardia, or uncoordinated contraction of the heart muscle cells.

A key purpose of this report is to examine the evidence that omega-3 fatty acids directly affect cell organelles such as cardiac ion channels, pumps, or exchange mechanisms involved in electrogenesis. The accompanying reports, entitled *Effects of Omega-3 Fatty Acids on Cardiovascular Disease Risk Factors* and *Effects of Omega-3 Fatty Acids on Cardiovascular Disease,* provide a review of the evidence from clinical studies of the effect of omega-3 fatty acids on arrhythmia and sudden death in humans.

Potential Impact of Omega-3 Fatty Acids on Arrhythmogenesis

As described above, cell organelles such as cardiac ion channels, pumps, currents, and exchange mechanisms are essential electrophysiological processes that ensure normal heart rate and coronary blood flow. These processes depend upon the concentration gradient of sodium, potassium, and calcium, and associated enzymes. Disruptions in these concentrations can lead to asynchronous contractility of the myocardium and result in arrhythmias. Clinically, the main causes of arrhythmia are ischemia, electrolyte disturbances, drugs, and underlying structural problems (e.g. bypass tracts). The physiologic mechanisms underlying these effects involve such mechanisms as ion channels and pumps and membrane currents.

Omega-3 LC PUFAs may exert an anti-arrhythmic effect on cardiac cells in several ways. For example, they can affect the adrenoceptors that transmit neuroendocrine messages sent by catecholamines. The omega-3 fatty acid, DHA, for instance, causes both a decrease in the production of cyclic adenosine monophosphate (cAMP), the main ß-adrenergic messenger, and an increase in chronotropic response or heart rate[11]. Omega-3 LC PUFAs also appear to act like another group of cardiovascular drugs, calcium channel blockers, by increasing intracellular calcium sequestration and interfering with receptor-operated calcium channels, influx [12].

Chapter 2. Methods

Overview

This evidence report on the effect of omega-3 fatty acids on cardiac electrogenesis and arrhythmias is based on a systematic review of the literature. To identify the specific issues central to this report, the Tufts-New England Medical Center (Tufts-NEMC) evidence-based practice center (EPC) held meetings and teleconferences with a Technical Expert Panel (TEP) formed for this project and with participants from the Agency for Healthcare Research and Quality (AHRQ) and the Office of Dietary Supplements (ODS). In addition, teleconferences with the Southern-California RAND (SC-RAND) and University of Ottawa (UO) EPCs were held to discuss common methodological issues associated with the production of the evidence report. A comprehensive search of the scientific literature was conducted to identify studies addressing the key questions. Evidence tables of study characteristics and results were compiled, and the methodological quality and applicability of the studies were appraised. Results were summarized with both qualitative reviews of the evidence and quantitative meta-analyses, as appropriate.

The TEP served in an advisory capacity for this project. It helped to refine key questions, identify important issues, and define parameters of the report. Additional domain expertise was obtained through consultation with lipid/nutrition experts.

Analytic Framework of This Evidence Report

We developed separate analytic frameworks to describe the relationship between omega-3 fatty acid intake and outcomes of interest in intact animal studies (Figure 2.1), intact animal/ isolated organ and cell studies (Figure 2.2), and isolated organ and cell studies (Figure 2.3). These frameworks served as a basis for the evidence review and highlight how omega-3 fatty acid intake impacts outcome measures/parameters and potential mechanisms associated with the following key questions:

- What is the evidence from whole animal studies that omega-3 fatty acids affect arrhythmogenic outcomes (and intermediate outcomes)?

- What is the evidence from cell culture and tissue studies (including animal and human cardiac tissue) that omega-3 fatty acids directly affect cell organelles such as cardiac ion channels, pumps, or exchange mechanisms involved in electrogenesis?

In whole animal studies (Figure 2.1), omega-3 fatty acids were fed to whole, intact animals as part of their diet or infused intravenously prior to the occurrence of the outcome of interest. The outcomes of interest in this context were induced arrhythmia, ventricular ectopic beats, ventricular and atrial fibrillation, and other measures of arrhythmia identified in the literature. Intermediate outcomes of interest included heart rate, coronary flow, and electrocardiogram (ECG) results such as QT interval prolongation.

In whole animal isolated organ and cell studies (Figure 2.2), omega-3 fatty acids were fed to whole, intact animals as part of their diet, and organs or cell tissues were subsequently excised from the animal for study. The outcomes of interest included induced arrhythmia, myocyte contraction and beating rate, and any other arrhythmogenic outcomes.

In "pure" isolated organ and cell studies (Figure 2.3), omega-3 fatty acids were applied directly to mammalian tissues or cultured cell lines or incorporated into the membrane of the mammalian tissues or cultured cell lines. The outcomes of interest included induced arrhythmia, myocyte contraction and beating rate, and any other arrhythmogenic outcomes.

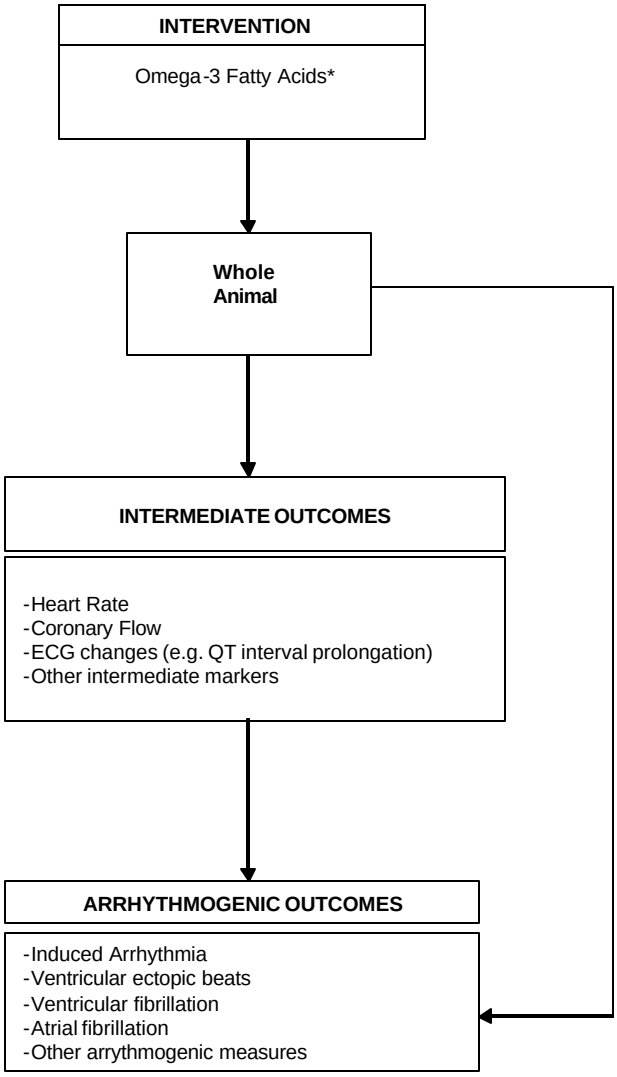
Potential mechanisms suggested by different investigators to explain the antiarrhythmic action of omega-3 fatty acids can be broadly classified into several categories (See list of Acronyms, Abbreviations, and Parameters). These include the effects of omega-3 fatty acids on:

- Contractile parameters (e.g. contractility)
- Basoelectromechanical parameters (e.g. action potential)
- Ion channels and pumps (e.g. calcium channels)
- Membrane currents (e.g. depolarizing current)
- Receptors (e.g. beta adrenergic)
- Membrane characteristics (e.g. fluidity and composition)
- Enzymes (e.g. sodium, potassium ATPases, adenosine triphosphatase)
- Eicosanoid system (e.g. prostaglandins)

Our focus in this report is limited to contractile parameters, basoelectromechanical parameters, ion pumps, channels, and membrane currents.

Figure 2.1 Analytic framework for animal studies

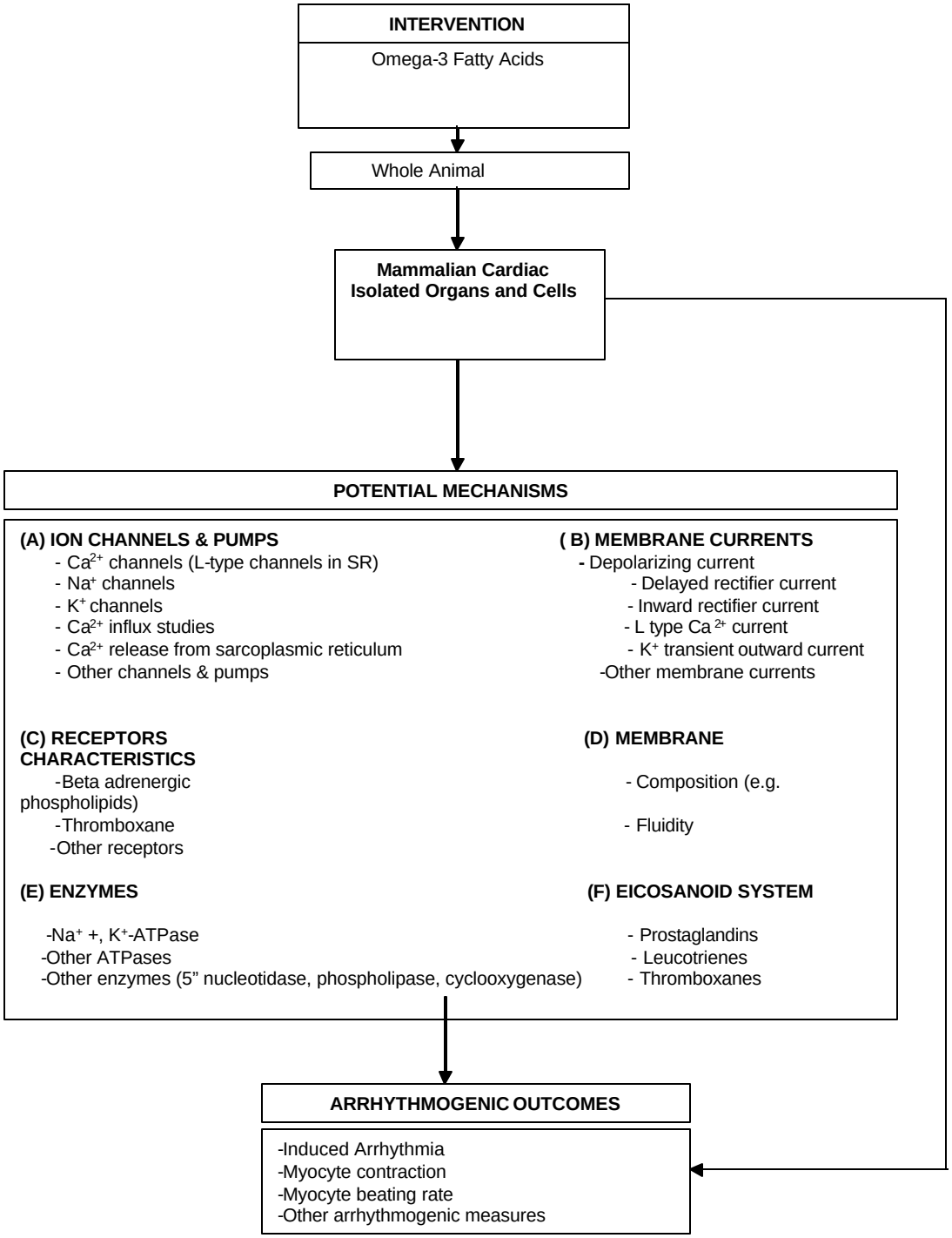
Question: What is the evidence from whole animal studies that omega-3 fatty acids affect arrhythmogenic outcomes (and intermediate outcomes)?



* ALA, EPA, DPA, DHA

Figure 2.2 Analytic framework for intact animal isolated organ and cell studies

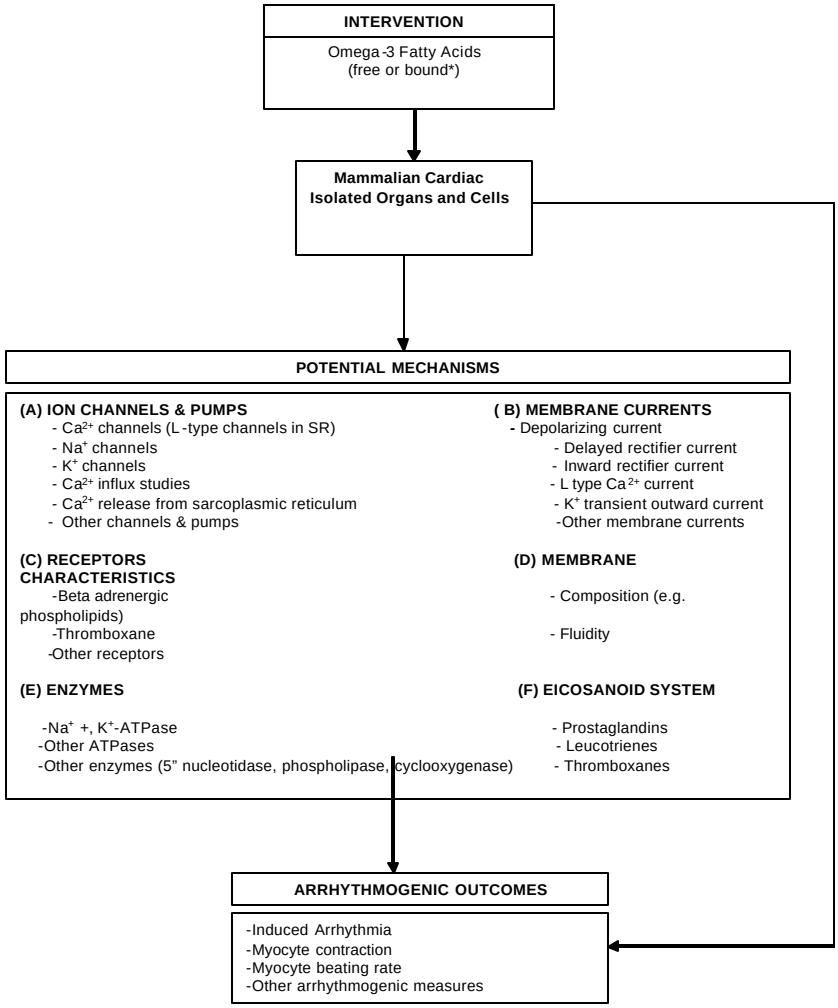
Question: What is the evidence from intact {intact, whole animal} cell culture and tissue studies (including animal and human cardiac tissue). that omega-3 fatty acids directly affect cell organelles such as cardiac ion channels, pumps, or exchange mechanisms involved in electrogenesis?



*omega-3 fatty acids (ALA, EPA, DPA, DHA) are fed to the animals as part of their diet, and organs, tissues or cells are subsequently excised from the animal for study.

Figure 2.3. Analytic framework for cell culture studies

Question: What is the evidence from cell culture and tissue studies (including animal and human cardiac tissue). that omega-3 fatty acids directly affect cell organelles such as cardiac ion channels, pumps, or exchange mechanisms involved in electrogenesis?



*omega-3 fatty acids (ALA, EPA, DPA, DHA) applied directly to (free) mammalian tissues or cultured cell lines or incorporated into the membrane (bound) of the mammalian tissues or cultured cell lines.

Literature Search Strategy

A comprehensive literature search was conducted to address the key questions. Relevant studies were identified primarily through search strategies conducted in collaboration with the UO EPC. Preliminary searches were conducted at the Tufts-NEMC EPC using the OVID search engine on the Medline database. The final searches used five databases including:

- Medline from 1966 to week 2 of February 2003
- PRE-MEDLINE from February 7, 2003
- Embase from 1980 to week 6 of 2003
- Biological Abstracts 1990 - December 2002
- Commonwealth Agricultural Bureau (CAB) Health from 1973 to December 2002

Subject headings and text words were selected so that the same set could be applied to each of the different databases with their varying attributes. Supplemental search strategies were conducted as needed. Additional publications were referred to us by the TEP and the other two EPCs.

A targeted search was conducted to retrieve articles that examined the effects of omega-3 fatty acids on cell organelles involved in electrophysiology. This search included in-vivo as well as in vitro animal studies. MeSH subject headings and text words were defined by reviewing key articles supplied by researchers and members of the TEP. In addition, citation analyses of key articles were conducted using the Science Citation Index database from the Institute for Scientific Information's Web of Science. Publications that cited the key articles were scanned for appropriateness and for additional subject headings or text words. These additional headings and text words were then added to those used in the search strategy.

Numbers for the final results of the database search strategies are approximate. Because the 5 main databases used in the search employ different citation formats, a number of duplicate publications were encountered. Although the UO EPC eliminated some of the duplicates, it was impossible to identify all of them. We eliminated additional duplicate publications as they were discovered. The database searches were updated regularly. The last update was conducted on April 18, 2003.

Study Selection

Abstracts identified through the literature search were screened using eligibility criteria defined to include all English language primary experimental studies that evaluated the impact of omega-3 fatty acids on arrhythmia, intermediate mechanisms of arrhythmia, and electrogenesis. Reports published only as letters or abstracts were excluded.

Articles associated with abstracts that passed these screens were retrieved and screened once more for eligibility. Inclusion and exclusion criteria used in this round of review are summarized below.

Inclusion Criteria

Studies were included if they examined the effect of omega-3 fatty acids on one of the following:

- Arrhythmia
- Adenosine triphophatase (ATPase, either Calcium, Sodium, Potasssium, or Magnesium)
- Beating rate
- Cardiac dynamics
- Cardiac or myocyte contraction
- Cardiomyocytes
- Cell organelles in cardiac tissue (sarcoplasmic reticulum or endoplasmic reticulum; mitochondria)
- Cell signaling
- Coronary perfusion pressure
- Cultured myocytes
- Electrogenesis in cardiac myocyte
- Electrophysiology
- Heart rate or rhythm
- Ion channels, pumps, currents, voltage dependant/sensitive channels (Calcium (Ca^{2+}), Sodium (Na^{+}), Potassium (K^{+}), K^{+} transient outward current, delayed rectifier current, inward rectifier current, L-type Ca^{2+} current or channel)
- Ischemia/ischemic reperfusion in heart
- Sudden cardiac death
- Ventricular fibrillation (VF)
- Ventricular fibrillation threshold (VFT)
- Ventricular ectopic beats (VEB)
- Ventricular premature beats (VPB); sometimes referred to as premature ventricular complex (PVC)

The TEP agreed that given the wide range and number of studies of potential relevance, prioritization of which to include was important. We therefore identified studies of the following mechanisms related to the antiarrhythmic action of omega-3 fatty acids but judged them not immediately relevant to the scope of the key questions to include in this report. Mechanisms excluded were:

- Eicosanoid production (prostaglandins, leucotrienes, thromboxanes)
- Enzymes (5'nuclotidase, phospholipase, cyclo-oxygenase)
- Receptors (ß-adrenergic, thromboxane)
- Membrane composition, fluidity, or phospholipids

For articles identified through the review, grounds for rejection included: non-mammalian animals or cell lines, no outcome of interest reported (see below), no omega-3 fatty acid intervention, review article, non-English article, and toxicology study/safety assessment. For each

study that was rejected, the reason(s) for rejection was noted. Basic information about all studies that addressed relevant outcomes was recorded.

Data Extraction

A standardized data extraction process was followed to ensure consistency across reviewers. Definitions for terms used in the extraction process were specified by consensus. As part of the training process, data extractors extracted data from 2 of the same studies to compare interpretations. After this process, each study was partially screened to determine whether it met eligibility criteria and addressed relevant outcomes. Studies deemed eligible were then fully extracted by a single reviewer. Issues and discrepancies encountered during the extraction process were addressed at weekly meetings.

For both animal and in vitro studies, general items extracted included country in which the experiment occurred, funding source, and sample size. Extraction of additional data relating to the intervention, intermediate outcomes, potential mechanisms, and arrhythmogenic outcomes was guided by the analytic framework described in Chapter 1.

For animal and animal in vitro studies, data extracted regarding the intervention included species of animal, animal characteristics, control and experimental diets (including detailed description of any omega-3 fatty acids), and dosage and duration of feeding or infusion. For animal studies, data extracted about intermediate outcomes included heart rate, coronary flow, and electrocardiogram (ECG) changes. Data extracted about arrhythmogenic outcomes included induced arrhythmia, ventricular ectopic beats, ventricular fibrillation, and atrial fibrillation.

For in vitro studies, data extracted regarding the intervention included species of animal, animal characteristics, cell line, sample sizes, number of experiments, detailed description of any omega-3 fatty acids, and whether the fatty acids were free (directly added to the cell culture medium) or bound (incubated with the fatty acid and incorporated into membrane phospholipid). Data extracted about potential mechanisms of arrhythmia included ion channels, ion pumps, and ion movement, as well as ion currents, contractility, and basoelectromechanical parameters.

Format for Reporting Evidence

We report the evidence in three forms: (1) *Evidence tables* offer a detailed description of the studies we identified that address each of the key questions. These tables provide detailed information about the study design, characteristics of the animal and in vitro model used in the study, inclusion and exclusion criteria, intervention or test evaluated, and outcomes. Where appropriate, we graded the studies according to the methodological quality, applicability, size, and the effect or test performance. (2) *Summary tables* report on each study in an abbreviated form using summary measures of the main outcomes. These tables were developed by condensing information from the evidence tables to provide a concise overview of study quality and results, and are designed to facilitate comparisons across studies. Summary tables include important variables including study size, omega-3 fatty acids evaluated in the study, study dosages and

duration, the animal model, outcomes, and methodological quality. (3) Additional tables were developed to provide an overall synthesis of information related to several key questions.

Methods of Analysis

For the whole animal studies, wherever feasible, we performed meta-analyses combining the results from individual experiments. It is important, however, to interpret results cautiously when combining data that are highly variable. We identified key measures and subgroups to construct random effects meta-analysis models [13].

For the isolated organ and cell studies, we frequently developed a qualitative summary of data presented in the articles. When possible, we report percentage changes in evidence tables. When a treatment group was compared to a control group, the difference in percentage change between the treatment group and control group was calculated. When one omega-3 fatty acid was compared to another fatty acid, we first report results of the comparisons to omega-6 fatty acids, followed by comparisons to monounsaturated fatty acids (MUFAs), then to saturated fatty acids (SFAs), and finally to other omega-3 fatty acids. In the summary tables, percentage changes are characterized as a statistically significant ($P<.05$) increase, decrease, improvement, or no change (i.e. change not statistically significant ($P>.05$).

Diet Classification

The criteria used to assess the methodological quality of animal studies are different from those used for human studies. Compared to human clinical trials, methods used to evaluate animal studies are not as advanced and there are no quality assessment rating schemes in widespread use. It is, however, important to stratify analyses, where possible, by the rigor of the study design and by the conduct, analysis, and reporting of the study. Since diet composition and the structure of the comparisons is a key aspect of study design in studies using intact animals fed different diets, we devised a four level categorization schema that is based on the fatty acid and/or level of fat contained in the comparison diet. The levels range from A to D, where the comparison diet in level A is most similar to eicosapentaenoic acid (EPA, 20:5 n-3) and decosahexaenoic acid (DHA, 22:6 n-3), and the comparison diet in level D is least similar. Specifically:

A. Omega-3 (fish, soybean, canola, linseed oils) vs. omega-6 (e.g. corn, safflower, sunflower oils) fatty acids. The omega-6 comparison oils have the longest fatty acid chains normally consumed by humans, and are most similar to EPA and DHA. They provide a similar level of dietary fat and have a similar number of double bonds.

B. Omega-3 fatty acids vs. MUFAs (e.g. olive oil). As with omega-6 comparison oils, MUFA oils have the longest fatty acid chains normally consumed by humans. They contain at least one double bond and provide a similar level of dietary fat.

C. Omega-3 fatty acids vs. SFA (e.g. butter, lard, palm oil, coconut oil, sheep fat). These saturated fatty acids provide a level of dietary fat in the comparison diet that is similar to the level obtained with omega-3 fatty acids.

D. Omega-3 fatty acids vs. control (e.g. standard chow). Standard chow is most different from the omega-3 enriched diet because no "counter-balancing" fatty acids are contained in this comparison diet.

In some studies, certain dietary comparisons conducted by the article authors were not relevant to this report. In such instances, only those components of the analysis that addressed the objectives of this report were extracted, using the scheme described above (order of comparison: omega-3 fatty acids to omega-6 fatty acids, MUFA, SFA, other omega-3 fatty acid).

Data Presentation

Data from the whole animal isolated organ and cell studies and the pure isolated organ and cell studies are presented in the evidence and summary tables in a specific order. Studies and/or comparisons are presented in the rows, and results or outcomes (e.g., contractile parameters [CP], basoelectromechanical parameters [BEP], ion pumps and ion movements, [IPIM], ion currents [ICU], and ion channels [ICH]) are presented in the table columns. For each outcome, the omega-3 fatty acid used, the dose, and the experimental condition under which the study was performed, is noted. Outcomes or results obtained under 'ambient' (no perturbation) conditions are presented first, followed by outcomes or results under other conditions. Presenting results in this order is similar to the order followed in the studies themselves: after observations were made in the ambient condition, specific blocking or facilitating agents (e.g., antagonists such as iosproteronol and agonists such as BAY8644 (BAY), respectively) were often introduced to investigate specific mechanisms (e.g., receptors) that are affected by the fatty acids. For example, isoproteronol was used in some studies to produce arrhythmia. This approach provides an understanding about which specific receptors are affected by omega-3 fatty acids and which omega-3 fatty acids might yield anti-arrhythmogenic effects. The parameter of interest in some studies is electrical current, which must be elicited by electrical stimulation. For the purposes of this report electrical stimulation is not considered an 'agent'.

Chapter 3. Results

In this chapter, we provide an overview of our literature search and discuss findings from the studies that met our search criteria. An overview of the literature search is presented first, followed by a review of whole animal studies, whole animal isolated organ and cell studies, and isolated organ and cell culture studies.

Literature Search Overview

Through the literature search, we identified 1,807 abstracts that met our search criteria. After screening the abstracts, we retrieved 274 articles. Of these, 184 were rejected after reviewing the full text. The reasons for rejection are as follows: no omega-3 fatty acids (30), not specific to arrhythmia (31), no cardiac cells (4), fatty acid composition or products only (34), other reasons (90). Details associated with the reasons for rejection are summarized in the reasons for rejection section. At the end of this process, 89 articles were accepted and reviewed.

For each class of study — whole animal studies, whole animal isolated organ and cell culture studies, and isolated organ and cell culture studies — we tabulated the outcomes/parameters measured by each investigator. Tables 3-1 to 3-3 summarize these parameters by species model and parameter.

Whole Animal Studies

A total of 26 whole animal studies (Tables 3-4 through 3-20 and Evidence Table 1) were reviewed. In 23 of the studies, omega-3 fatty acid supplements were added to the animals' food for a variable duration of time before experimental protocols for induced arrhythmias were implemented. In the remaining 3 studies, fatty acids were infused intravenously as a treatment to prevent induced or spontaneous arrhythmias. In the pre-fed route, dietary fatty acids must be incorporated into an animal's cell membrane before they can influence cell function and/or rehabilitation. In contrast, when omega-3 fatty acids are directly injected into an animal's blood stream, they exist and function in free form. The results of these two types of studies will be discussed separately, since their presumed physiological mechanisms differ. A summary of the 26 whole-animal studies is shown in Table 3-1.

Individual summary tables were created to show the effects of omega-3 fatty acids on various arrhythmic outcomes. Studies were grouped first by outcomes, then by species, and finally by experimental protocols (or mechanisms) for induced arrhythmias. Within each table, comparisons were first clustered into alpha linolenic acid (ALA, 18:3 n 3) oils or fish oils, and then sorted by the dose of omega-3 fatty acids. Frequently, studies had more than one comparison and used more than one experimental protocol. As a result, some studies appear multiple times in one table (once for each comparison group) or appear in several different tables.

In general, the arrhythmic outcomes assessed were defined consistently across the 26 whole-animal studies with the exception of the definition for arrhythmia score, which varied

somewhat across studies. The following arrhythmic outcome measures and general definitions were used in the original studies:

- **Ventricular Tachycardia (VT)**: A run of four or more consecutive ventricular premature beats [14].

- **Ventricular Fibrillation (VF)**: A signal for which individual QRS deflections can no longer be distinguished from one another (implying morphological instability) and for which a rate can no longer be measured [14].

- **Ventricular Premature Beats (VPB)**: Isolated ventricular premature beats are generally defined as discrete and identifiable premature QRS complexes (premature in relation to the P wave) [14].

- **Arrhythmia Score (AS)**: A hierarchical scale of 0 to 9 during occlusion as most described by Curtis et al., 1987 [15], and during reperfusion using a slightly modified version of the scale as described by McLennan et al., 1988 [16].

- **Infarct Size (IS)**: The under-perfused ischemic regions determined by dye exclusion and expressed as a percentage of wet weight in both ventricles [16]. In the studies examined for this report, infarct size reflects myocardial tissue that has sustained damage due to the ischemia procedures that were used to induce arrhythmias.

We performed meta-analyses for each of the outcomes. In these analyses, fish oils and ALA oils were analyzed separately and in combination.

In the following sections, the 23 pre-fed studies are discussed first and are grouped according to the comparison substance. Studies comparing omega-3 polyunsaturated fatty acids (PUFAs) to omega-6 PUFAs are presented first, followed by studies comparing omega-3 PUFAs to a-linolenic acid, monounsaturated fatty acids (MUFAs), saturated fatty acids (SFAs), and no treatment. The 3 studies that infused free form omega-3 fatty acids are reviewed at the end of the *Whole Animal Studies* section.

Studies Comparing Pre-Fed Omega-3 PUFAs to Omega-6 PUFAs

This section summarizes 13 studies that compared pre-fed omega-3 PUFAs to pre-fed omega-6 PUFAs (see Table 3-1 and Evidence Table 1). In each study, the same amount of experimental and control oil was added to each animal's basic diet. Therefore, all comparisons have iso-caloric intake from fat. The dose of omega-3 fatty acids ranged from 0.4 to 3.7g/100g. Fish oils (menhaden, tuna fish oils, or MaxEPA---a commercial preparation of EPA), soybean oil, or canola oil were used as the source of omega-3 PUFAs in the experimental groups, while controls were fed sunflower seed oil, corn oil or safflower oil. The effects of omega-3 PUFAs on arrhythmia deaths, ventricular fibrillation, ventricular premature beats, arrhythmia scores, infarct size, and length of time in sinus rhythm are reviewed below.

Effect on incidence of arrhythmia deaths. Seven studies in rats (Table 3-4) and one study in monkeys (Table 3-6) reported arrhythmia deaths. In the rat studies, investigators looked for deaths in ischemia-reperfusion-induced arrhythmias in 12 comparisons. In the monkey study, investigators looked for deaths after induced arrhythmias in 1 comparison (Table 3-6).

Meta-analyses of risk ratio of total deaths in ischemia-reperfusion-induced arrhythmias. As shown in Table 3-4, 12 comparisons in 7 studies were included in the meta-analyses. The 7 studies involved 150 rats that were fed omega-3 PUFAs and 152 rats fed omega-6 PUFAs. In all but one [17] of the studies, deaths during reperfusion after an ischemia procedure were monitored. Two studies [18,19] also looked for deaths that occurred during the ischemia procedure. They all found that deaths occurred only during the ischemia procedure; no deaths occurred during reperfusion in either the omega-3 PUFA or control groups The ischemia deaths in these two studies were combined into total deaths for ischemia-reperfusion-induced arrhythmia.

Of the 12 comparisons, 5 compared ALA oils to omega-6 PUFA oils (Figure 1). The combined risk ratio of deaths in ischemia-reperfusion-induced arrhythmias in these 5 comparisons was 1.2 (95% CI: 0.51-2.6). There was no statistically significant heterogeneity between studies.

The other 7 comparisons were combined to assess the effects of fish oils on deaths in ischemia-reperfusion-induced arrhythmias (Figure 2). The combined risk ratio of deaths in these 7 comparisons was 0.47 (95% CI: 0.23-0.93). There was no statistically significant heterogeneity between studies. However, the significantly reduced risk ratio of deaths was due to a single study [20] as shown by a sensitivity analysis (Table 3-5). When this study was removed, the combined risk ratio of deaths became 0.64 (95% CI: 0.19-2.1).

A separate meta-analysis combined comparisons involving ALA with comparisons involving eicosapentaenoic acid (EPA, 20:5 n-3) plus decosahexaenoic acid (DHA, 22:6 n-3). The overall risk ratio of deaths in this analysis was 0.68 (95% CI: 0.40-1.2).

Deaths from ventricular fibrillation in monkeys. One study examined total VF deaths— which combined deaths in the control condition, ischemia model, and isoproterenol model — among marmoset monkeys (Table 3-6). For the purpose of our evidence review, we evaluated only the results from a comparison between 16 monkeys fed fish oil and 13 monkeys fed sunflower seed oil. The fish oil and sunflower seed oil diets both had 12% weight-by-weight (w/w) of total fat or 29% kcal of fat. The fish-oil diet contained 2.8g/100g EPA plus DHA. The animals were fed for 30 months in both studies. No VF deaths occurred in the monkeys that were fed fish oil, while 3 deaths (23%) occurred in those fed sunflower seed oil.

Effects on incidence of ventricular tachycardia. Eight studies, representing 21 comparisons, reported the incidence of VT among rats fed omega-3 PUFA oils vs. those fed omega-6 PUFA oils (Table 3-7 and Table 3-8). In 10 of the comparisons, the incidence of VT in ischemia-induced arrhythmias was monitored (Table 3-7). In the other 11 comparisons, the incidence of VT during reperfusion-induced arrhythmias was monitored (Table 3-8). Only ischemia-induced VT was assessed in 2 [21,22] of the 8 studies. The remaining 6 studies assessed both ischemia-induced and reperfusion-induced VT.)

Meta-analyses of risk ratio of ventricular tachycardia in ischemia-induced arrhythmias. As shown in Table 3-7, 10 comparisons in 6 studies were included in the meta-analyses. Of the 248

rats used in the studies, 126 were in the omega-3 PUFA groups and 122 were in the omega-6 PUFA control groups.

Among the 10 comparisons, 4 examined the effects of ALA vs. omega-6 PUFA oils on the incidence of VT in ischemia-induced arrhythmias (Figure 3). The dose of ALA ranged from 0.4 to 5.2g/100g. The combined risk ratio of deaths was 0.82 (95% CI: 0.65-1.0). There was no statistically significant heterogeneity between studies.

The other 6 comparisons were combined to evaluate the effects of fish oils (EPA plus DHA) on the incidence of VT in ischemia-induced arrhythmias (Figure 4). The combined risk ratio of deaths in this analysis was 0.49 (95% CI: 0.29-0.83). The studies were heterogeneous. Sensitivity analysis did not show that any single study had a dominating effect.

A separate meta-analysis combined comparisons involving ALA with comparisons involving EPA plus DHA. In this meta-analysis, the overall risk ratio of VT in ischemia-induced arrhythmias was 0.70 (95% CI: 0.53-0.92).

Meta-analyses of risk ratio of ventricular tachycardia in reperfusion-induced arrhythmias. As shown in Table 3-8, 11 comparisons in 7 studies were included in these meta-analyses. Of the 257 rats used in the studies, 128 were in the omega-3 PUFA groups and 129 were in the omega-6 PUFA control groups.

Among the 11 comparisons, 5 examined the effects of ALA vs.omega-6 PUFA oils on the incidence of VT in reperfusion-induced arrhythmias (Figure 5). The combined risk ratio of deaths was 1.1 (95% CI: 0.73-1.6). The studies were heterogeneous.

The other 6 comparisons were combined to evaluate the effects of fish oils (EPA plus DHA) on the incidence of VT in reperfusion-induced arrhythmias (Figure 6). The combined risk ratio of deaths was 0.68 (95% CI: 0.50-0.91). There was no statistically significant heterogeneity between studies.

In the meta-analysis that combined comparisons involving ALA with comparisons involving EPA plus DHA, the overall risk ratio of VT in reperfusion-induced arrhythmias was 0.85 (95% CI: 0.65-1.1).

Effects on incidence of ventricular fibrillation. Nine studies in rats with 22 comparisons (Table 3-9 & Table 3-10), and 3 studies in monkeys with 9 comparisons (Table 3-11), reported the incidence of VF in induced arrhythmias. All the rat studies used ischemia-reperfusion models. In the monkey studies, arrhythmias were induced by electrical stimulation in normal or ischemic conditions and/or with the injection of isoproterenol.

In the rat studies, the incidence of VF in ischemia-induced arrhythmias was monitored in 8 comparisons (Table 3-9), while in the other 14 comparisons, the incidence of VF during reperfusion after an induced-ischemia procedure was monitored (Table 3-10). (Four [20-23] of the nine rat studies monitored only the incidence of reperfusion-induced VF. The remaining 5 studies monitored the incidence of both ischemia-induced and reperfusion-induced VF.)

Meta-analyses of risk ratio of ventricular fibrillation in ischemia-induced arrhythmias. As shown in Table 3-9, a total of 8 comparisons from 6 studies were included in the meta-analyses. Of the 176 rats used in the studies, 90 were in the omega-3 PUFA groups and 86 were in the omega-6 PUFA control groups.

Among the 8 comparisons, 3 examined the effects of ALA vs. omega-6 PUFA oils on the incidence of VF in ischemia-induced arrhythmias (Figure 7). The combined risk ratio of deaths was 0.95 (95% CI: 0.56-1.6). There was no statistically significant heterogeneity between studies.

The other 5 comparisons were combined to evaluate the effect of fish oils on the incidence of VF in ischemia-induced arrhythmias (Figure 8). The combined risk ratio of deaths was 0.21 (95% CI: 0.07-0.63). There was no statistically significant heterogeneity between studies.

In the meta-analysis that combined ALA comparisons and EPA plus DHA comparisons, the overall random-effect risk ratio of VF in ischemia-induced arrhythmias was 0.69 (95% CI: 0.41-1.24).

Meta-analyses of risk ratio of ventricular fibrillation in reperfusion-induced arrhythmias. As shown in Table 3-10, a total of 14 comparisons in eight studies were included in these meta-analyses. Of the 312 rats used in the studies, 155 were in the omega-3 PUFA groups and 157 were in the omega-6 PUFA control groups.

Among the 14 comparisons, 6 examined the effects of ALA vs. omega-6 PUFA oils on the incidence of VF in reperfusion-induced arrhythmias (Figure 9). The combined risk ratio of deaths was 0.84 (95% CI: 0.52-1.3). The studies were heterogeneous.

The other 8 comparisons were combined to evaluate the effects of fish oils on the incidence of VF in reperfusion-induced arrhythmias (Figure 10). The combined risk ratio of deaths was 0.44 (95% CI: 0.25-0.79). There was no statistically significant heterogeneity between studies.

In the meta-analysis combining ALA comparisons and EPA plus DHA comparisons, the overall random-effect risk ratio of VT in reperfusion-induced arrhythmias was 0.85 (95% CI: 0.65-1.1).

Ventricular fibrillation and ventricular fibrillation threshold in induced arrhythmia among monkeys. Table 3-11 shows results from 3 studies that compared monkeys fed fish oils to controls fed sunflower seed oil (omega-6 PUFA), and that examined the incidence of VF and ventricular fibrillation threshold (VFT) in induced arrhythmia. The dose of EPA plus DHA ranged from 1.8 to 2.8g/100g. The feeding duration ranged from 16 weeks to 30 months. Three different arrhythmia-induction protocols were used. In the first protocol, arrhythmias were induced by electrical stimulation in the control condition. In the second, arrhythmias were induced 5 minutes after an ischemia procedure, and in the third, arrhythmias were induced 30 minutes after restoration of coronary blood flow and during the infusion of isoproterenol. The three arrhythmia-induction protocols were not independent of each other, that is, the same monkeys underwent the series of experimental procedures in sequence. Thus, the cumulative effects of induced arrhythmias must be considered. (Note also be noted that the same group of investigators from one laboratory authored all three studies).

For each of the arrhythmia induction protocols, the investigators compared the proportion of monkeys from the fish oil group that experienced inducible VF to the proportion in the sunflower seed oil group that experienced VF. In the first protocol (electrical stimulation in control condition), the investigators found no difference between groups. In the second protocol (electrical stimulation five minutes after an ischemia procedure), 2 of the 3 studies found no difference in the proportion of monkeys that had inducible VF [24,25]. One study [26], however, reported that while no VF was inducible in the monkeys fed fish oil, VF was induced in 13% of the monkeys fed sunflower seed oil . In the third protocol (electrical stimulation during the infusion of isoproterenol), VF was induced in 30% to 50% of the monkeys fed fish oil compared to 77% to 100% of the monkeys fed sunflower seed oil.

Ventricular fibrillation thresholds (VFTs) were measured only among the VF inducible monkeys. In 2 studies[24,26], VFTs remained unchanged in both groups in all conditions. However, one study [25] found that VFTs were significantly increased among monkeys that were fed fish oil relative to those fed sunflower seed oil in all conditions (note that an increased threshold indicates a desirable outcome).

Effects on ventricular premature beats. As shown in Table 3-12, 7 studies with 16 comparisons evaluated the number of VPBs in ischemia-induced and/or reperfusion-induced arrhythmias. Rats were used in all 7 studies. No consistent results were found in the studies comparing rats fed ALA oils (soybean, linseed or canola oils) to rats fed omega-6 PUFA oils. However, studies comparing rats fed fish oils to rats fed omega-6 PUFA oils suggest that rats fed fish oils might have reduced numbers of VPBs in ischemia-induced and/or reperfusion-induced arrhythmias relative to rats fed omega-6 PUFA oils.

Effects on arrhythmia scores or severity of arrhythmias. As shown in Table 3-12, 8 studies with 18 comparisons evaluated the arrhythmia scores associated with the ischemia-induced and/or reperfusion-induced arrhythmias. Rats were used in all studies. More severe arrhythmias are associated with higher scores.

No consistent results were found in studies that compared rats fed ALA oils (soybean, linseed or canola oils) to rats fed omega-6 PUFA oils. However, when rats fed fish oils were compared to rats fed omega-6 PUFA oils, the studies found that most of the fish oil fed rats had less severe ischemia-induced and/or reperfusion-induced arrhythmias than the omega-6 PUFA fed rats.

Effects on infarct size. Infarct size, or size of the ischemic region, was evaluated in only 2 studies [16,27]. The results showed no significant difference in the infarct size between rats fed omega-3 PUFA oils and rats fed omega-6 PUFA oils (Table 3-12).

Effects on length of time in sinus rhythm. As shown in Table 3-12, 3 studies with 7 comparisons evaluated length of time in sinus rhythm (TSR) in ischemia-induced and/or reperfusion-induced arrhythmias. Rats were used in all studies. One study [27] that compared rats fed linseed oil (rich in ALA) to rats fed corn oil found no significant difference in TSR. In the same study, however, TSR was significantly increased in rats fed fish oil compared to rats fed corn oil. Two other studies compared rats fed fish oils to rats fed omega-6 PUFA oils. These studies found no significant difference in TSR in ischemia-induced and/or reperfusion-induced arrhythmias [19,28].

Studies Comparing Pre-fed Omega-3 Long-Chain PUFAS to a-Linolenic Acid

Two studies directly compared omega-3 long-chain PUFAs (EPA and DHA) to ALA (Table 3-13). Both studies found a non-significant reduction in the incidence of VT and VF in ischemia-induced or reperfusion-induced arrhythmias in rats fed fish oils compared to those fed soybean or linseed oils (rich in ALA) [23,27]. Abeywardena et al. found that no deaths occurred in rats fed fish oil, while 11% of rats fed soybean oil died from ischemia-reperfusion-induced arrhythmias [23]. The results also showed that rats fed fish oil had fewer numbers of VPBs and less severe arrhythmias as indicated by arrhythmia score than did rats fed soybean oil. However, none

of these results were statistically significant. In addition to the incidence of VT and VF, Isensee et al. [27] also examined the infarct size and the length of time in normal sinus rhythm. The results showed no difference in infarct size between rats fed a fish oil diet and those fed a linseed oil diet after 20 minutes of ischemia. The length of time in normal sinus rhythm was almost 50% longer in rats fed fish oil compared to rats fed linseed oil.

Indirect comparisons between omega-3 long chain PUFAs (EPA and DHA) and ALA based on meta-analysis are described in the Discussion (Chapter 4).

Studies Comparing Pre-fed Omega-3 PUFAs to MUFAs

One study [29] compared the anti-arrhythmic effects of PUFAs to those of MUFAs (see Evidence Table 1). In this study, rats that were fed EPA, DHA, or a mixture of EPA and DHA were compared to rats that were fed olive oil. All animals used in the study were male, spontaneously hypertensive strains (n=10 per group). All synthesized diets contained 5% total fat, which represented 12% of available energy as fat. The rats underwent a common surgical procedure to induce myocardial ischemia after eating synthetic diets for 5 weeks. Results showed that DHA and EPA plus DHA significantly reduced the incidence and severity of ventricular arrhythmias as indicated by the arrhythmia score; however, EPA alone had no effect. Ventricular fibrillation occurred in 80% of the rats who were fed olive oil, 70% of those fed EPA, 20% fed DHA, and 10% fed the mix of EPA plus DHA. Compared to the controls, the incidence of VF was significantly lower in the DHA-fed rats ($P<.01$) and in the rats fed the mix of EPA plus DHA ($P<.01$). However, VF was not significantly lower in rats fed the EPA diet.

Studies Comparing Pre-fed Omega-3 PUFAS to Saturated Fatty Acids

As shown in Table 3-1, we analyzed 5 studies that compared omega-3 PUFAs to saturated fatty acids. In each study, experimental and control oils were added to the animals' basic diets in equal amounts (see Evidence Table 1). Therefore, all comparisons reflect iso-caloric intake from fat. Fish oils and sardine or mackerel oils were used as the source of omega-3 PUFAs in the experimental groups, while controls were fed coconut, lard, sheep peri-renal fat, or butter. The dosages of EPA plus DHA were 0.6g/100g [30], 2.9g/100g [31], 5.5g/100g [32] and 5%kcal [33,34].

Effects on incidence of arrhythmia deaths. As shown in Table 3-14, deaths in ischemia-reperfusion-induced arrhythmias were monitored in 2 studies. In 1 study [33], rabbits fed fish oil corresponding to a dose of EPA plus DHA of 5.2g/100g were compared to controls fed coconut oil. The animals were fed for 12 weeks before arrhythmias were induced. In one arm of the study, animals were subjected to 10 minutes of ischemia followed by one hour of reperfusion. Three deaths (25%) were observed among the 12 rabbits fed fish oil, compared to three deaths (36%) among the 14 rabbits fed coconut oil. Two of the deaths in both groups occurred during reperfusion. In another arm of the study, rabbits were subjected to 1 hour of ischemia followed by 4 hours of reperfusion. Six deaths (43%) were observed among the 14 rabbits fed fish oil, compared to 8 deaths (53%) in the 15 rabbits fed coconut oil. About 50% of the deaths occurred during ischemia and 50% occurred during reperfusion in both groups in this arm.

In another study [30], 13 piglets were fed either 9%w/w lard fat (n=6) or 4.5%w/w mackerel oil plus 4.5%w/w lard fat (n=7) for 16 weeks. The corresponding dose of EPA plus DHA in the group

fed mackerel oil plus lard fat was 0.6g/100g. Defibrillation was unsuccessful in one piglet from the mackerel plus lard oil group. This piglet died of ventricular asystole during the fifth reperfusion.

Effects on incidence of ventricular tachycardia. As shown in Table 3-15, 2 studies examined the incidence of VT in ischemia-reperfusion-induced arrhythmias. One of these studies was in rats [35], and 1 was in piglets [30]. In the rat study, 7 (35%) of the 20 rats fed fish oil developed VT, compared to 14 (70%) of the 20 rats fed sheep peri-renal fat ($P<.05$).

In the piglet study, the incidence of VT was 29% (n=7) and 17% (n=6) in piglets fed mackerel oil and lard fat, respectively. All VT events occurred during the ischemia procedure.

Effects on incidence of ventricular fibrillation. As shown in Table 3-16, 3 studies examined the incidence of VF in ischemia-reperfusion-induced arrhythmias. Two of these studies were in rats [34,35] and 1 was in piglets [30]. Both rat studies found a significantly reduced incidence of VF in ischemia-reperfusion-induced arrhythmias among rats fed fish oil compared to rats fed saturated fats.

In the piglet study, the incidence of VF was 43% (n=7) in piglets fed mackerel oil, while no piglet fed lard fat developed VF in ischemia-reperfusion-induced arrhythmias. In the same study, programmed electrical stimulation was performed to induce VF in another 20 piglets, 10 in the mackerel-oil group and 10 in the control group. The incidence of VF was not reported, but VFTs were measured in control condition and during 15 minutes of ischemia. The threshold current for VF induction was reduced in all dietary groups during ischemia but remained significantly higher in the mackerel-oil-fed group than in the saturated-fat-fed group.

Effects on ventricular premature beats. As shown in Table 3-17, 3 studies examined the number of VPBs in schemia-reperfusion-induced arrhythmias. One of these studies was in rabbits [33], 1 was in piglets [30], and 1 was in rats [35]. In the rat study, the number of VPBs during ischemia was significantly reduced among rats fed fish oil compared to rats fed sheep-perirenal fat. In the piglet study, the incidence of VPBs during ischemia did not differ between the groups. However, during reperfusion the piglets fed mackerel oil had significantly fewer VPBs compared to those fed lard fat. In the rabbit study, there were no significant differences in the incidence of VPBs between rabbits fed fish oil and those fed coconut oil during ischemia or reperfusion. However, rabbits that died from arrhythmias were excluded from the analyses, and more rabbits died in the control group than in the experimental group. Thus, the true effects were underestimated.

Effects on arrhythmia scores or severity of arrhythmias. None of the studies that compared the arrhythmic effects of omega-3 PUFAs and saturated fatty acids reported arrhythmia scores as an outcome.

Effects on infarct size. None of the studies that compared the arrhythmic effects of omega-3 PUFAs and saturated fatty acids reported infarct size as an outcome.

Effects on length of time in sinus rhythm. None of the studies that compared the arrhythmic effects of omega-3 PUFAs and saturated fatty acids reported length of time in sinus rhythm as an outcome.

Studies Comparing Pre-fed Omega-3 PUFAS to No Treatment Controls

A total of 4 studies were included in this analysis (Table 3-1). As shown in Evidence Table 1, omega-3 PUFA oils were added to the diet of animals in the experimental groups, while controls were maintained on basic diets. As a result, energy intake from fat was higher in the experimental groups than in the control groups. Dogs were used in all studies. MaxEPA, menhaden oil, or EPA esters were used as the source of omega-3 PUFAs in the experimental groups, while controls were fed standard dog chows (Oriental Yeast Co. or Friskies® Dinner). The dose of EPA plus DHA was 3.3%kcal [36] and 1.0g/100g [37] in the two fish-oil studies. The dose of EPA was 1.0g/100g in both of the EPA-ester studies [38,39].

Effects on incidence of arrhythmia deaths. Two studies [36,39] that evaluated the incidence of arrhythmia deaths (Table 3-18) compared dogs fed EPA and/or DHA to no treatment controls. In one study, no significant difference was found in the incidence of sudden death after induced-coronary thrombosis. [36] The other study found no deaths due to VF in the 10 dogs fed 1.0g/100g EPA ester, although five VF deaths (33%) occurred in the 15 untreated control dogs ($P<.05$). [39].

Effects on incidence of ventricular tachycardia and/or ventricular fibrillation. The incidence of VT and/or VF in induced arrhythmias was evaluated in a study of 30 dogs. [38]. Fifteen dogs were fed standard dog chow plus 1.0g/100g EPA ester for 8 weeks. Fifteen untreated control dogs were fed standard dog chow for 8 weeks. An ischemia-induced arrhythmia model was used in 10 experimental dogs and 10 controls. A digitalis-induced arrhythmia model was used in 5 dogs from each group. A fatal dose of digoxin (0.025 mg/kg/min) was administrated intravenously over a 60-second period immediately after coronary artery ligation.

There was no difference in the incidence of VF in ischemia between the groups. Two dogs in each group (20% vs. 20%) developed VF within 3 hours after coronary ligation. All 10 dogs that underwent digitalis-induced arrhythmias developed VT or VF. However, the VT or VF did not occur until at least 25 minutes after the administration of digoxin in the dogs fed EPA ester, while the events occurred about 10 to 15 minutes within administration of digoxin in the untreated control dogs.

Effects on ventricular premature beats. As shown in Table 3-19, 2 studies [36,38] examined the number of VPBs in induced arrhythmias. Both studies found that dogs fed EPA and/or DHA had fewer VPBs compared with untreated controls.

Effects on arrhythmia scores or severity of arrhythmias. One study [38] evaluated the arrhythmia score in ischemia-induced arrhythmias (Table 3-19), and found that the arrhythmia score obtained within 3 hours after coronary ligation was significantly reduced by EPA-supplementation. Dogs fed EPA esters for 8 weeks had significantly less severe ischemia-induced arrhythmias than the no treatment controls ($P<.01$).

Effects on infarct size. Infarct size, or size of the ischemic region, was evaluated in 3 studies (Table 3-19) [36,37,39]. All 3 studies showed that dogs fed EPA and/or DHA had a decrease in the infarct size in either electrical-stimulation-induced or ischemia-reperfusion-induced arrhythmias

compared to untreated controls. However, areas at risk of arrhythmias were not significantly different between groups.

Effects on length of time in sinus rhythm. None of the studies that compared the arrhythmic effects of omega-3 PUFAs vs. untreated controls reported length of time in sinus rhythm as an outcome.

Anti-arrhythmic Effects of Free Omega-3 Fatty Acids

Three studies examined the effects of intravenously infused omega-3 fatty acids on ischemia-induced or spontaneous arrhythmias (Table 3-20). The fatty acids were infused in their free form bound to albumin. Dogs were used in all studies. Controls received infusions of saline or buffer, or of soybean lipid emulsion with 7-8% ALA. Cardiac function was monitored by ventricular electrocardiography. Because cardiac response was similar among the control groups, data for control dogs were combined if 2 groups of controls were used.

Effects on incidence of ventricular tachycardia and ventricular premature beats. One study [40] evaluated the incidence of VT in spontaneous arrhythmias in 8 dogs (Table 3-20). The dogs were first injected with control buffer. Data obtained after this injection served as controls. After all hemodynamic parameters had completely recovered, the same protocol was used to infuse the dogs with various doses of ALA: 1 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, or 60 mg/kg. No VT or VPB events were observed when infusing the control buffer or when infusing up to 10 mg/kg of ALA. However, at doses of 20 mg/kg, 30 mg/kg, and 60 mg/kg of ALA, the incidence of VT was 13%, 38%, and 63%, respectively. The effects of ALA on the number of VPBs was similar. However, possible cumulative effects are of concern in this study since the experiments were not independent of one another.

Effects on incidence of ventricular fibrillation. As shown in Table 3-20, 2 studies [41,42] evaluated the incidence VF in exercise-plus-ischemia-induced arrhythmias. The results showed fish-oil emulsion, or albumin-bound EPA-, DHA-, or ALA-concentrates significantly reduced the incidence of VF.

Whole-Animal Isolated Organ and Cell Studies

In this section, we present the results of 21 studies that examined the effects of omega-3 fatty acids in isolated organs and cells from whole animals. In these studies, omega-3 fatty acids were fed to whole, intact animals as part of their diet, and organs or cell tissues were subsequently excised from the animal for study. The effects of omega-3 fatty acids on the following parameters are discussed: contractile parameters, basoelectromechanical parameters, ion pumps and ion movements, ion currents, and ion channels. Tables 3-20 through 3-24 and Evidence Table 2 contain the results for this section.

Contractile Parameters

Eight studies evaluated the effect of diets enriched with omega-3 fatty acids on contractile parameters such as heart rate, contraction rate, contraction amplitude, diastolic and systolic cell length, percent cell length, post-rest potentiation, and cardiac work. All studies used rat models. The developmental stage of the rats, however, varied considerably (2 weanling; 2 young adults; 3 adults; 1 aged). See Table 3-21.

Heart rate. Under ambient conditions and in the absence of any agent, 3 studies showed that fish oil or EPA/DHA supplementation did not change heart rate [43-45]. One study showed that in the presence of the arrhythmogenic agent lipopolysaccharide (LPS), fish oil significantly decreased heart rate compared to a safflower oil diet [43]. One study examined the effect of cod liver oil supplementation on heart rate under various conditions. In the absence of nor-adrenalin under high oxygenation, there was a significant decrease in heart rate, but there was no change in the presence of nor-adrenalin. In the presence of nor-adrenalin under hypoxic conditions, there was a significant decrease in heart rate. Upon re-oxygenation, there was no change. [46]. See Table a 3-21.

Contractility. Two studies by the same author compared the effects of fish oil supplementation, safflower oil, and lard on contraction rate induced by isoproteronol (ISO) and free radical generating system (FRGS) [47,48]. Both studies found a significant decrease in contraction rate among the fish oil group. Another study compared the effects of fish oil and safflower oil on force of contraction, maximum rate of rise of contraction, and maximum rate of relaxation. This study found no change in any of the parameters in the presence of saline, but found a significant increase in all parameters in the presence of lipopolysaccharide [43]. One study measured force-velocity relationship characteristics following consumption of an N-3 fatty acid diet vs. an N-6 diet and showed no change [49]. See Table 3-21.

Ionotropic parameters. One study examined the effect of fish oil versus lard treatment on diastolic and systolic cell length, percent cell length, and post-rest potentiation, and showed no change in these parameters [48]. Another study measured amplitude of contraction under various experimental conditions. In the absence of nor-adrenalin under high oxygenation, there was a significant decrease in amplitude of contraction, but there was no change in the presence of nor-adrenalin. In the presence of nor-adrenalin under hypoxic conditions, there was a significant decrease in amplitude. Upon re-oxygenation, there was no change [46]. See Table 3-21.

Cardiac work. One study compared the effects of linseed oil treatment and sunflower oil treatment on cardiac work and reported no difference between the two groups [50]. See Table 3-21.

Basoelectromechanical Parameters

Three studies examined the effect of omega-3 fatty acids on basoelectromechanical parameters in whole animal isolated organs and cells. One study used a rat model and showed that supplementing a high fat diet with fish oil significantly reduced the ventricular effective refractory period [45]. Another rat model study reported no change in developed or resting tension in the isolated perfused heart after cod liver oil supplementation [51]. The third study used a rabbit model and showed no effect of dietary fish oil compared to safflower oil on the ventricular effective

refractory period, absolute refractory period, relative refractory period, or epicardial or endocardial monophasic action potential [52].
See Table 3-22.

Ion Pumps and Ion Movement

Fourteen studies examined the impact of omega-3 fatty acid enriched diets on ion pumps and ion movement (IPIM) in whole animal isolated organs and cells. Three studies used mouse models, 8 used rat models, 1 used rabbit models, 1 used pig models, and 1 used a canine model. See Table 3-23.

Pump activity. Eight studies examined either calcium-magnesium ATPase or sodium-potassium ATPase activity in isolated organs and cells from whole animals. Among the 3 studies that used mouse models, one study compared diets enriched with EPA ester or DHA to a diet containing safflower oil and found no change in sarcoplasmic reticulum calcium-magnesium ATPase activity with either the EPA ester or DHA ester diet [53]Croset, 1989b]. A second mouse study compared a diet rich in fish oil to one rich in corn oil and found a significant decrease in sarcoplasmic reticulum calcium-magnesium ATPase activity with the fish oil diet[54]. The third mouse study showed that, compared to a standard chow diet, supplementation with graded doses of DHA ester did not affect calcium-magnesium ATPase activity in the SR, but at low doses it significantly increased calcium-magnesium ATPase in the cardiac myocyte. At a higher dose, however, there was no change [55]. Two studies used a rat model. One compared a fish oil diet to a corn oil diet and used a graded dose of ATP and ionomycin, and measured sarcoplasmic reticulum calcium-magnesium ATPase, calcium ATPase, and magnesium ATPase. This study found significant decreases in these parameters [56]. A study using a canine model, reported significant increases in cardiac calcium-magnesium ATPase with EPA ester supplementation [38]. Three studies (2 rat and 1 canine model) all reported no change in sodium-potassium ATPase activity with an omega-3 fatty acid diet, regardless of dosage or agent used [38,57,58]. One study using a pig model reported significant increases in calcium pumping ATPase activity after consumption of a fish oil vs. a lard-enriched diet both under ambient and ischemia-reperfusion conditions [59]. See Table 3-23.

Cytosolic calcium influx. Two studies using rat models measured cytosolic calcium influx. One reported a significant increase in cytosolic calcium influx under ischemic conditions with a cod liver oil diet [51]. Another study compared fish oil to canola oil and reported no change under ambient conditions in cytosolic calcium (Ca^{2+}) influx [60]. See Table 3-23.

Cytosolic calcium efflux. Only one study compared cod liver oil supplementation to a standard chow diet using a rat model under ischemic reperfusion conditions. That study reported no change in cytosolic calcium efflux under ischemia reperfusion conditions [51]. See Table 3-23.

Cytosolic calcium content. Three studies using rat models examined the effect of fish oil supplementation on cytosolic calcium content. In comparison to an omega-6 or saturated fatty acid diet, fish oil supplementation demonstrated no effect under ambient conditions in any of the studies [32,33,47]. Two of these studies examined the effect of fish oil under ischemic/reperfusion conditions. One study found no change [33], while the other reported a significant decrease in

cytosolic calcium content which was more pronounced in aged (vs. younger) rats [61]. See Table 3-23.

Sarcoplasmic reticulum calcium content. Three studies (one mouse and two rat models) examined the effect of fish oil supplementation on sarcoplasmic reticulum calcium content. Two of the studies showed a significant decrease in sarcoplasmic reticulum calcium content. One of the 2 studies compared ALA or EPA or DHA ester to a safflower oil control, while the other compared fish oil supplementation to a corn oil diet [53,56]. The third study compared fish oil supplementation to a diet enriched with saturated fats and reported no difference in caffeine or 2,4-Di-tert-butylhydroquinone (DBHQ)-induced alterations in sarcoplasmic reticulum calcium content with fish oil supplementation compared to one enriched with saturated fats [47]. See Table 3-23.

Sarcoplasmic reticulum calcium uptake. Two studies (one mouse and one rat model) compared the effects of fish oil supplementation vs. corn oil on sarcoplasmic reticulum calcium uptake. Both studies showed a significant decrease in sarcoplasmic reticulum calcium uptake among rats receiving fish oil supplementation [54,56]. Another study used a rat model to compare fish oil supplementation to a saturated fat diet. This study reported a significant increase in sarcoplasmic reticulum calcium exchanger or sarcoplasmic reticulum efflux induced by DBHQ or isoproteronol [47] among the rats receiving fish oil. One study comparing the effect of fish oil supplementation to a standard chow diet demonstrated no change in sarcoplasmic reticulum calcium transport activitiy using a rat model[62]. See Table 3-23.

Ion Currents

Two studies examined the effect of omega-3 fatty acid diet supplementation on ion currents in isolated organs and cells from whole animals. Both studies used rat ventricular myocytes, and both studies compared a fish oil diet to a high fat diet [48,63]. See Table 3-24.

Sodium currents. One study measured sodium currents (I_{NA}) and reported no change in either activation or inactivation parameters [48]. See Table 3-24.

Transient outward currents.
One study measured transient potassium outward currents (I_{to}) and reported no change in either activation or inactivation parameters [48]. See Table 3-24.

Voltage dependent L-type calcium current. One study measured voltage dependent L-type calcium current (ICa.L) and observed no change in activation parameters, inactivation parameters, or amplitude of voltage dependent L-type calcium current.[63]. See Table 3-24.

Ion Channels

Two studies evaluated the effect of omega-3 fatty acid diet supplementation on ion channels in whole animal isolated organs and cells. Rat models were used in both studies. One of the studies measured in ventricular crude sarcolemma preparations the binding site affinity and affinity (K_d)

of [^{3}H] nitrendipine for the calcium channels. There was no change reported in either B_{max} or K_d attributable to cod liver oil in adult rats. A similar result was observed in aged rats. When aged rats were compared to adult rats, there was a significanty lower K_d in the aged rats [64]. A second study comparing fish oil to a high fat diet assessed the binding characteristics of the phenylalkylamine (PAA) receptor with verapamil and those of the benzonthiazepine (BT) receptor with diltiazem and reported no change on the parameters of the calcium current-voltage (I_{Ca}–V) curves [63]. See Table 3-25.

Isolated Organ and Cell Culture Studies

In this section, we present the results of 39 studies that examined the effects of omega-3 fatty acids on isolated organs and cells extracted from whole animals. Twenty-nine of these studies used rat models, 1 used a mouse model, 2 used guinea pig models, 2 used dog models, 1 used a ferret model, 1 used a pig model, and 1 used a cat model. Two studies used both rat and guinea pig models. Tissues and organelles extracted for analysis included the whole heart, ventricular or atrial cardiomyocytes, sarcolemmal or microsomal vesicles, and myocardial or ventricular mitochondria. The omega-3 fatty acids tested in these studies included ALA, EPA, DHA, or their combination. The omega-3 fatty acids were applied either directly to the cell culture medium (free) or incubated with the cells to allow incorporation into membrane phospholipids (bound). Each row of the summary tables represents a comparison using the following factors: study, diet, free or bound fatty acid, dosage, experimental condition (ambient, hypoxia, reoxygenation) or agent used. Tables 3-26 through 3-31 and Evidence Table 3 contain the results for this section.

Contractile and Arrhythmogenic Parameters

This section summarizes 22 studies that examined the effect of omega-3 fatty acids on arrhythmogenic and contractile parameters in isolated organs or cells. In 11 of these 22 studies, the omega-3 fatty acids were free, and in 9 studies the cells were bound with the fatty acids. Two studies employed both approaches. Nineteen studies used rat models, 2 used guinea pig models, and 1 used both a rat and guinea pig model. See Table 3-26.

Arrhythmias. Seven studies examined the effect of omega-3 fatty acids on arrhythmias. Arrhythmias were defined as spontaneous or asynchronous contractions induced by various agents. Four of the studies using rats were from the same group of collaborators and demonstrated that free EPA or DHA significantly prevented or terminated the proportion of arrhythmias induced by ouabain, calcium, lysophosphatidylcholine (LPC), palmitoylcarnitine (PTC), or eicosanoids [65-67,67,68]. Another study by the same collaborative group examined the effect of free and bound EPA or DHA in a rat model, and demonstrated that free but not bound omega-3 fatty acids were effective in terminating induction of arrhythmias [69]. Another study using a rat model showed that bound DHA significantly decreased the proportion of arrhythmias induced by nor-adrenaline and timolol (TIM) [70]. A study using a guinea pig model showed that free EPA (sodium salt) at a low dosage did not have an effect on antigen-induced arrhythmia but produced a significant decrease in the proportion of induced arrhythmias at a high dosage [71]. See Table 3-26.

Contractility. Eighteen studies examined the effect of omega-3 fatty acids on contractility parameters such as contraction rate (spontaneous or induced), contraction frequency, electrical automaticity/excitability (EA) , diastolic length (DL), twitch amplitude (TA), velocity of shortening /diastolic length (VS/DL), and twitch size (TS) both in the presence and absence of several arrhythmogenic agents. Fourteen studies used rat models, 2 studies used guinea pig models, and 2 studies used both rat and guinea pig models. See Table 3-26.

In 13 of these studies the effects of the omega-3 fatty acids were studied compared to a control group; in 1 study the comparison group was a saturated fatty acid, in 2 studies the comparison group was either control or an omega-6 fatty acid, and in 2 studies (by the same author) the comparison group was another omega-3 fatty acid. The results are discussed based on the comparison group and agent used. See Table 3-26.

In the contractility studies that tested the effect of free ALA, EPA, DHA, or a combination compared to control in the absence of any agent, 3 showed no effect [65,72,73], while 3 showed a decrease [66,74,75]. The following arrhythmogenic agents were examined: ouabain, nitrendipine, Bay8644 (BAY), isoproteronol, LPC, dibutyryl cyclic adenosine monophosphate (dBcAMP), eicosanoids, high extracellular calcium, and cholera toxin. All studies reviewed, regardless of species used, demonstrated a decrease in contractility or a protective effect of the omega-3 fatty acids in blocking the negative response induced by the agents [65-68,72,73,76,77,78]. One study also showed that DHA blocked the inhibitory effect of nitrendipine on myocyte contraction but not the inhibitory effect of verapamil and diltiazem on myocyte contraction [65]. See Table 3-26.

One study examined the effect of free DHA versus the saturated fatty acids docosanoic acid and stearic acid in the presence of LPC or isoproteronol in a rat model and observed a significant decrease in both spontaneous and asynchronous contractility [79]. Two studies examined the effect of a combination of either free ALA+EPA [73] or bound EPA+DHA [80] compared to an omega-6 fatty acid and found no difference in contractility in the absence of an arrhythmogenic agent. In the presence of arrhythmogenic agents (isoproteronol and phenylephrine [PHE]), one study showed no effect of free ALA+EPA [73], while the other study observed a significant increase with bound EPA+DHA [80]. See Table 3-26.

In 2 studies [11,81] of bound EPA compared to bound DHA (omega-3 vs omega-3), there was no effect on frequency of spontaneous contractions in the absence of an agent or with PHE. However, in the presence of an agent such as ISO or dBcAMP, bound EPA was significantly more effective than bound DHA in reducing the frequency of spontaneous contractions. See Table 3-26.

Three studies also examined the effect of methylated (m.e.) or ethylated (e.e.) free EPA or DHA on contractility. Two of these studies were performed using rat models and showed that free EPA e.e. in the absence of an agent, or free DHA m.e. in the presence of ISO, had no effect on contractility [66,76]. The third study, which used a guinea pig model, showed that free DHA methyl ester (m.e.) significantly increased calcium-induced calcium release (CICR) contractions but not voltage-sensitive release mechanism (VSRM) contractions [82]. See Table 3-26.

One study examined the effect of free DHA on DL, TA, and VS/DL in a rat model and showed no effect in the absence of an agent or ISO, but produced a blockade with the addition of nitrendipine or BAY [61]. See Table 3-26.

Two studies examined the effect of omega-3 fatty acids on twitch size, and both used rat and guinea pig models [77,83]. A decrease in twitch size with free EPA and/or free DHA was observed in both guinea pig studies. In the studies using rats, 1 study observed an increase in twitch size with EPA or DHA at concentrations between 1-7.5µm, and decreases in twitch size with concentrations

>10µm [83]. In the other rat study, 5 µm of EPA significantly decreased twitch size [77]. See Table 3-26.

Inotropic parameters. Three studies examined the effect of omega-3 fatty acids on inotropic parameters. One study using a rat model reported that neither free EPA nor DHA had an effect on amplitude of contraction [66]. Free EPA significantly increased resting cell length in another study using a rat model [74]. A third study using bound EPA with a rat model showed no change in amplitude but a significant increase in amplitude with ouabain [72]. See Table 3-26.

Other contractility parameters. Seven studies using rat models (3 by the same investigator[11,81,84] examined the effect of bound omega-3 fatty acids on the following contraction parameters: 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). See Table 3-26.

Two of these studies examined the effect of bound omega-3 compared to bound omega-6 fatty acids under 3 conditions — ambient, hypoxia, and reoxygenation — and showed no effect on the contractility parameters that were investigated [85]. Four studies (2 from the same laboratory) compared bound EPA to DHA and found no difference in their effects on CD_{20}, CD_{80}, –Cmax, and +Cmax, regardless of the agents used to induce arrhythmia [80,81,84,86]. One study compared bound ALA+EPA to omega-6 fatty acids and reported no difference in CD_{80} and –Cmax but found a significant increase in +Cmax [73] with ALA+EPA. The presence of ISO did not alter the effect of ALA+EPA on these parameters. See Table 3-26.

Basoelectromechanical Parameters

This section summarizes 9 studies (4 from the same group of collaborators) [11,84-86] that examined the effects of omega-3 fatty acids on basoelectromechanical parameters in isolated organs and cells. Seven of these studies used rat models. One study used both a rat and guinea pig model, and 1 used a cat model. Free omega-3 fatty acids were used in 3 rat studies, in the study using both rat and guinea pig models, and in the study using the cat model. Bound omega-3 fatty acids were used in 4 of the studies using rat models. See Table 3-27.

The single study that used a feline model examined the effect of free ALA on four basal electric parameters not measured by any of the other researchers — intra-atrial conduction time, atrioventricular conductance time, atrial functional refractory period, and functional refractory period of the atrioventricular conducting system [87]. No changes were observed in any of these parameters. See Table 3-27.

Action potential. Six studies using rat models examined the effect of omega-3 fatty acids on the action potential. One reported an increase [88] with free EPA compared to a control, while another study, also using free EPA, reported a significant decrease in both the action potential and the frequency of the action potential [89]. See Table 3-27.

In the presence of 3 different agents (sodium and timolol [TIM], isoproteronol, and ouabain), bound DHA was shown to significantly decrease the action potential compared to control. No change was observed in the absence of an agent [70]. Two studies compared bound synthesized medium for omega-3 group (SM3) to bound synthesized medium for omega-6 group (SM6) and

reported no change in the action potential under ambient, hypoxic, and reoxygenated conditions [84,85]. See Table [3-27].

A study that compared bound EPA to bound DHA also found no difference in effect [86]. See Table 3-27.

Action potential amplitude. Seven studies examined the effect of omega-3 fatty acids on the amplitude of the action potential. All studies used rat models. Two studies showed that 5-10µM of free EPA and/or DHA did not affect the action potential amplitude (APA) compared to control [88,89], but concentrations >10-50 µM showed a significant decrease [88]. One study compared the effect of bound DHA relative to control and reported a significant increase in action potential amplitude using EPA [70]. See Table 3-27.

Two studies examined the effects of omega-3 fatty acid combinations (SM3) versus omega-6 fatty acids (SM6), under varying conditions. Both showed no change in APA under ambient conditions and a significant decrease in APA under hypoxic conditions. Under the reoxygenation condition, however, the results differed; one study reported no change [85] and the other reported a significant increase in action potential amplitude [11]. See Table 3-27.

Two studies compared the effect of bound EPA to bound DHA and found that EPA significantly increased APA compared to DHA [11,86]. See Table 3-27.

Action potential duration at 40% depolarization. Four studies using rat models examined the effect of omega-3 fatty acids on the action potential duration at 40% polarization. One study reported an increase in this parameter in the presence of both free EPA and free DHA compared to control [88]. See Table 3-27.

Two studies compared bound SM3 to bound SM6 under varying experimental conditions, 1 reported no change under all 3 conditions [84], while the other reported a significant decrease in action potential duration at 40% polarization under hypoxic conditions with SM3, but no change under ambient or reoxygenation conditions for [85]. See Table 3-27.

One study comparing bound EPA to bound DHA did not find a differential effect on this basal electromechanical parameter [86]. See Table 3-27.

Action potential duration at 80% depolarization. Five studies using rat models and 1 study using both a rat and guinea pig model examined the effect of omega-3 fatty acids on the action potential duration at 80% polarization (APD_{80}). One study using free EPA (10µM) compared to control, reported a significant decrease in the action potential duration [89]. Similarly, another study reported a dose dependent decrease in action potential duration at 80% polarization with EPA concentrations >10µM but an increase with EPA concentrations between 1-7.5µM [83]. The same authors also used a guinea pig model and reported that EPA was effective in decreasing action potential duration at 80% polarization at concentrations between 1-20µM. See Table 3-27.

Two studies compared bound SM3 to bound SM6 under varying experimental conditions, 1 reported no change under all 3 conditions [84], while the other reported a significant decrease in action potential duration at 80% polarization under hypoxic conditions, but no change under ambient or reoxygenation conditions [85]. See Table 3-27. Two studies compared bound EPA to bound DHA and observed no effect on the action potential [11,86]. See Table 3-27.

Maximum rate of depolarization. Six studies using rat models examined the effect of omega-3 fatty acids on the maximum rate of depolarization (V_{MAX}) of the action potential. One

study showed a decrease in V_{max} with either free EPA or free DHA compared to control [88]. See Table 3-27.

Two studies compared bound SM3 to bound SM6 under varying experimental conditions. One reported no change under any of the 3 conditions [84], while the other reported a significant increase in V_{max} under ambient conditions, but observed no change under either hypoxic or reoxygenated conditions [85]. See Table 3-27.

Two studies compared bound EPA to bound DHA and found no difference in V_{MAX} [11,86]. See Table 3-27.

Maximum diastolic potential. Four studies using rat models examined the effect of omega-3 fatty acids on the maximum diastolic potential (MDP). See Table 3-27.

Two studies compared bound SM3 to bound SM6 under varying experimental conditions, and observed that SM6 did not affect MDP under ambient and hypoxic conditions [84,85]. However, under reoxygenation conditions, one study showed an improvement [85] while the other showed no change [84]. Two studies compared bound EPA to bound DHA and both reported no change in MDP [11,86]. See Table 3-27.

Overshoot potential. Four studies (all by the same collaborative group) using rat models examined the effect of omega-3 fatty acids on the overshoot potential (OS). A study comparing bound SM3 to bound SM6 reported no effect on OS [85]. Another study also compared bound SM3 to SM6 but under varying experimental conditions, and found that SM3 did not affect OS differently from SM6 under ambient conditions, but significantly decreased OS under hypoxic conditions and significantly increased OS during reoxygenation [84]. See Table 3-27. Two studies comparing bound EPA to bound DHA reported that EPA significantly increased OS compared to DHA [11,86]. See Table 3-27.

Other basoelectromechanical parameters. In a cat model infusion of ALA in the presence of indomethacin there was no change in the following basoelectrical parameters such as AC, AVC, ARP, and AVRP.

Ion Pumps and Ion Movements

This section summarizes 13 studies that examined the effects of omega-3 fatty acids on ion pumps and ion movements in isolated organs and cells. In 10 of these studies, the omega-3 fatty acids were applied directly in free form, and in 2 studies the cells were incubated with the fatty acids to allow incorporation into membrane phospholipids (bound). In 1 study both approaches were used. Nine studies used rat models, 2 used canine models, 1 used a pig mode1 used both a rat and guinea pig model. See Table 3-28.

Pump activity. One study, which used a rat model, examined the impact of bound EPA on pump activity (sodium-potassium ATPase). This study reported no effect in the presence of ouabain or bumetanide (BUME), or with a combination of these two agents [72]. See Table 3-28.

Cytosolic calcium influx. Three studies examined the effect of omega-3 fatty acids on cytosolic calcium influx. The first used a rat model and reported that free EPA decreased cytosolic calcium influx [90]. In the second study, free DHA blocked the effect of nitrendipine and BAY on

cytosolic calcium influx [61]. Another study using a rat model examined the effect of bound EPA or bound DHA in the presence of several agents and found that DHA blocked the ouabain-induced increase in cytosolic calcium influx. Both EPA and DHA blocked the nitrendipine (NIT)-induced decrease, ouabain+nitrendipine-induced decrease, BAY+nitrendipine-induced decrease, and the BAY-induced increase in cytosolic calcium influx [65]. See Table 3-28.

Cytosolic calcium efflux. One study using a rat model examined the effect of free EPA on cytosolic calcium efflux in the presence of either calcium or caffeine and demonstrated no effect [90]. See Table 3-28.

Cytosolic calcium content. Seven studies examined the effect of omega-3 fatty acids on cytosolic calcium content. One study directly compared the effect of acute and chronic exposure to free DHA on cytosolic calcium content[91]. This study showed that both acute and chronic exposure to DHA were effective in decreasing the magnitude of increase in cytosolic calcium content induced by an agent (potassium chloride [KCl]) or under an anoxic condition. See Table 3-28.

While 2 of the studies [74,90] showed that free EPA decreased cytosolic calcium content, the other 4 studies showed that neither free nor bound EPA or DHA had an effect on cytosolic calcium content [61,72]{Vitelli, 2002 100059 /id}[78]. In the presence of various agents (NIT, BAY, ISO, KCl, Endothelin-1, Ca^{2+} free Krebs Ringer bicarbonate buffer [KRB], Doxorubicin [DXR] and caffeine), free or bound EPA and DHA blocked the alterations in cytosolic calcium induced by these agents. See Table 3-28.

Sarcoplasmic reticulum calcium content. Only 1 study using a rat model examined the effect of free EPA on sarcoplasmic reticulum calcium content and reported an increase in the presence of caffeine [74]. See Table 3-28.

Sarcoplasmic reticulum calcium uptake. No studies were identified that specifically studied the effect of omega-3 fatty acids on sarcoplasmic reticulum uptake of calcium. See Table 3-28.

Sarcoplasmic reticulum calcium release. Two studies examined the effect of omega-3 fatty acids on sarcoplasmic reticulum calcium release. One of these studies used both rat and guinea pig models and found that free EPA significantly decreased the sarcoplasmic reticulum calcium release [77]. Another study using a rat model found that free DHA increased sarcoplasmic reticulum calcium release in the presence of DXR and caffeine [92]. See Table 3-28.

Sodium-calcium and sodium-hydrogen exchangers. There were 3 studies that examined the effect of omega-3 fatty acids on sodium-calcium and sodium-hydrogen exchange. Two of these studies used a canine model and were by the same investigator. Both reported that free ALA increased sodium-calcium exchange. The other study used a pig model and showed that free ALA did not affect sodium-hydrogen exchange [93,94]. However, there was a dose-dependent decrease attributable to EPA at 50 and 100µM, but not at 10 and 25µM. DHA also decreased the sodium-hydrogen exchange. See Table 3-28.

Other ion pump and ion movement outcomes. One study using a rat model showed that free EPA decreased calcium transients [95]. Two studies by the same investigator using canine models

showed a significant increase in passive sarcoplasmic reticulum calcium efflux attributable to free ALA [93,94]. One study using a pig model showed that free EPA or free DHA had no impact on passive sodium influx [96]. See Table 3-28.

Ion Currents

This section describes 12 studies that examined the effect of free omega-3 fatty acids on ion currents in isolated organs or cells; 1 study used a mouse model, 7 of the studies used rat models; 1 used a guinea pig model, 1 used a ferret model, and 2 used both rat and guinea pig models. See Table 3-29.

Sodium current. Three studies examined the effect of free omega-3 fatty acids on sodium current parameters (I_{Na}) including amplitude, the current-voltage relation, and activation and inactivation parameters. The first study, using a rat model, demonstrated a significant shift to more positive potentials in the voltage dependence of activation, and a significant shift to more negative potentials in the inactivation of the sodium current, using ALA, EPA, or DHA [97]. A study using both rat and guinea pig models found a dose-dependent decrease in peak amplitude of the sodium current with both EPA and DHA [83]. In another study using a rat model, a significant time, dose, and voltage-dependent decrease of the sodium current was observed using ALA, EPA, or DHA. There was, however, no change in the current-voltage relationship and activation or inactivation parameters of the sodium current [98]. See Table 3-29.

Transient potassium outward current. Four studies examined the effect of free omega-3 fatty acids on transient potassium outward current (I_{to}) parameters, including amplitude, frequency, and the time constant of transient potassium outward current. The first of these studies used a rat model and showed that both EPA and DHA decreased I_{to} amplitude and the time constant of I_{to} inactivation, and increased the I_{to} delay [88]. The presence of indomethacin did not modify this effect, suggesting that the effects of the omega-3 fatty acids are not related to their cyclo-oxygenase products. In the second of these studies using a rat model, there was a dose dependent decrease in I_{to} [83] . In the third study, which also used a rat model, EPA significantly decreased the frequency and significantly increased the amplitude of I_{to} [90]. In the last study, which used ferrets, ALA, EPA, or DHA significantly decreased I_{to} amplitude (ALA<EPA<DHA) [99]. See Table 3-29.

Voltage dependent L-type calcium current. Six studies examined the effects of free omega-3 fatty acids on the voltage dependent L-type calcium I_{Ca}.L currents. Using a rat and guinea pig model, one study found there was a dose-dependent decrease in voltage dependent L-type calcium current with both EPA and DHA [83]. Similarly, in a rat and guinea pig model study comparing EPA to standard chow, there was a significant decrease in voltage dependent L-type calcium current [77]. In a rat model study, both EPA and DHA decreased the amplitude of voltage dependent L-type calcium current [74]. In a study examining the effect of various agents on voltage dependent L-type calcium current, DHA increased the amplitude of the current in the presence of nitrendipine. DHA also blocked the BAY K8644-induced increase in voltage dependent L-type calcium current amplitude, but did not change the amplitude in the presence of isoproteronol or in the absence of an agent [61]. In another study using a rat model, significant time, dose, and voltage-dependent decreases in voltage dependent L-type calcium current were observed in the

presence of ALA, EPA, or DHA, along with a negative shift in the voltage dependent L-type calcium current inactivation curve [95]. In a study of guinea pigs using methylated DHA, a significant increase in voltage dependent L-type calcium current was observed [82]. See Table 3-29.

Delayed rectifier potassium current. Two studies examined the effect of free omega-3 fatty acids on delayed rectifier potassium current (I_K). One study observed a decrease in I_K using EPA in both rat and guinea pig models [83], and the other study, using a ferret model, also showed a significant decrease with either ALA, EPA, or DHA [99]. See Table 3-29.

Inward rectifier potassium current. Four studies examined the effect of free omega-3 fatty acids on inward rectifier potassium current (I_{KI}). One study using a mouse model showed no effect of DHA [100]. Another, using a rat model, showed no effect of either EPA or DHA [88]. A third study using EPA with rat and guinea pig models showed a decrease in rectifier potassium current [83]. The ferret model study showed no change using ALA, EPA, or DHA [99]. See Table 3-29.

Ultra rapid potassium current. Two studies examined the effect of free omega-3 fatty acids on ultra rapid potassium current (I_{KUR}). One using a mouse model showed a significant decrease in I_{KUR} with 30µM of DHA[100]. The other study, using a rat model, showed a significant decrease in I_{KUR} at dosages above 20µM of EPA or DHA but no effect with an EPA dose of 5-10µM [88]. See Table 3-29.

Ion Channels

Three studies examined the effect of omega-3 fatty acids on ion channels in isolated organs or cells; 1 study used a mouse model [100] and 2 used rat [65,101]. In the mouse model study, the investigators examined the effect of free DHA on activity of the cloned Kv1.5 potassium channel, and observed that while DHA significantly blocked this activity, free ALA had no effect. See Table 3-30.

One of the rat model studies examined nitrendipine binding to putative dihydropyridine-sensitive calcium channels and reported that both bound EPA and bound DHA significantly decreased both the high and low affinity binding sites (B_{max}) as well as the K_d values of those binding sites. With DHA, the high affinities were so diminished that they were undetectable [65]. The other study using a rat model examined the effect of bound EPA on the number of sodium channels per cell and showed no change; however, the combination of EPA with mexiletine significantly reduced the number of sodium channels and blocked the mexiletine-induced increase in sodium channel expression [101]. See Table 3-30.

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.

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Listing of Excluded Studies

Animal and isolated organ and cell culture rejected articles

1. No omega-3 fatty-acid treatment or inntervention (30 articles)

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List of Acronyms, Abbreviations, and Parameters

Acronyms and Abbreviations

Abbreviation	Definition
*	P<.05
**	P<.01
***	P<.001
A	Amplitude
AA (20:4 n-6)	Arachidonic acid
Ac	Activation parameter
AC	Intra-atrial conduction time
AHRQ	Agency for Healthcare Research and Quality
AI	Adequate Intake
ALA (18:3 n-3)	Alpha-linolenic acid
Amb	Ambient
AP or APR	Action potential rate
APA	Action-potential amplitude
APD_{40}	Action-potential duration at 40% depolarization
APD_{80}	Action-potential duration at 80% depolarization
Apo	Apoprotein
APS	Active pump sites
APT	Action potential threshold
AR	Arrhythmia
ARAr	Areas at risk of arrhythmias
ARP	Functional refractory period of the atrium
AS	Arrhythmia scores
ASC	Asynchronous contraction
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
AVC	Atrioventricular conductance time
AVRP	Functional refractory period of atrio-ventricular conducting system
B	Basal
B	Blocked (when used with arrythmias)
BAY	Bay K8644
BEP	Basal electromechanical parameters
B_{max}	High and low affinity binding sites
BMI	Body mass index
BP	Blood pressure
BSA	Bovine serum albumin
BT	Benzonthiazepine calcium current-voltage (I_{Ca}–V) curves
BUME	Bumetamide
BW	Lipoxygenase inhibitor
C_{20}	Contraction coupling delay (ms)
Ca	Calcium
Ca^{2+}	Calcium
CAB	Commonwealth Agricultural Bureau
cAMP	Cyclic adenosine monophosphate
CAFF	Caffeine
CaFlu	Calcium intermittent fluctuations
CCTR	Cochrane Central Register of Controlled Trials
CD_{20}	Contraction duration at 20% relaxation (ms)
CD_{80}	Contraction duration at 80% relaxation (ms)
CICR	Calcium induced contractile response
CL	Cell Length

Abbreviation	Definition
CLO	Cod liver Oil
+Cmax	Cell shortening velocity
$-C_{max}$	Relaxation time
CO	Corn Oil
Contra	Contractility or beating rate (beats/min)
COX	Cyclo-oxygenase
CP	contractile parameters
CSF II	Continuing Food Survey of Intakes by Individuals 1994-1998
CRP	C reactive protein
Ctrl	Control
CVD	Cardiovascular disease
Cys	Cytosolic
D	Dietary supplement company (in evidence table)
D	Duration (in summary table)
D	Decreased (when in footnote of table)
dBcAMP	Dibutyryl cyclic adenosine monophosphate
DBHQ	2,4-Di-tert-butylhydroquinone
DCL	Diastolic cell length
DD	Dose dependent
Dep APT	Depolarizing action potential threshold
df/dt	Maximum rate of rise of contraction
-df/dt	Maximum rate of rise of relaxation
DHA (22:6 n-3)	Decosahexaenoic acid
DHAe	DHA esters
DHPA	3H-dihydroalprenolol
Dia	Diastolic
DIL	Diltiazem
DL	Diastolic length
DM	Diabetes mellitus
DPA (22:5 n-3 or n-6)	Docosapentaenoic acid
DRI	Dietary References Intakes
DTS	Dense tubular system
DXR	Doxorubicin
EA	Electrical automaticity/excitability
EAR	Estimated Average Requirement
ECG	Electrocardiogram
EC50	That concentration needed to produce a 50% effect
e.e.	ethylated
EFA	Essential fatty acid
endo	endocardial
EPA (20:5 n-3)	Eicosapentaenoic acid
EPA-e	EPA esters
EPC	Evidence-based Practice Center
Epi	epicardial
ET-1	Endothelin-1
ETYA	Eicosatetraynoic acid
F	Frequency
FAC	Fatty acid composition
FDA	Food and Drug Administration
FO	Fish Oil
FOC	Force of contraction
FRGS	Free radical generating system

Abbreviation	Definition
FVR	Force-velocity relationship (Vmax, initial muscle length, maximum extent of twitch muscle shortening, time to peak shortening, positive peak of the normalized force derivative of the fully isometric twitch, total isometric force normalized per cross-sectional area, time to peak force)
G	Government
GLA (18:3 n-6)	Gamma linolenic acid
HC	High Cholesterol
HDL	High density lipoprotein
HF	High fat
HTN	Hypertension
Hy RMP	Hyperpolarizing
I	Industry
I	Increased (when in footnote of table)
$I_{Ca.L}$	Voltage dependent L-type Ca^{2+} current/inward Ca^{2+} current/Ca^{2+} sparks
I_{Ca}–V	Calcium current-voltage
ICH	Ion channel
$I_{Cl.Ca}$	Caffeine and Neurokinin A elicited Ca^{2+} dependent Cl^- current
ICU	Ion currents
IC50	That concentration that produces a 50% reduction in the effect
I_K	Delayed rectifier K^+ current
I_{KI}	Inward rectifier K^+ current or tail current
I_{KUR}	Ultra rapid potassium current
IL	Interleukin
InAc	Inactivation parameter
In	Ionomycin
I_{Na}	Sodium current
INDO	Indomethacin
InsP	Inositol phosphate
IOM	Institute of Medicine
IP	Inotropic Parameters
IPIM	Ion pumps and ion movement
IS	Infarct size
ISO	Isoproteronol;
I_{SUS}	Outward K^+ current
I_{to}	Transient K^+ outward current or initial outward current
K^+	Potassium
KCl	Potassium chloride
Kd	Affinity
KRB	Krebs Ringer bicarbonate
L	Membrane leakiness
L	Resting cell length (inotropic measure in contractile parameter table)
LA (18:2 n-6)	Linoleic acid
LC PUFA	Long-chain polyunsaturated fatty acid
LD	Lactate dehydrogenase
LDL	Low density lipoprotein
LIN	Linseed Oil
LP	Lipoprotein
LPC	lysophosphatidylcholine
LPS	lipopolysaccharide
LT	Leukotriene
LVH	Left ventricular hypertrophy
Mag	Magnitude
MAP	Monophasic action potential duration
MDP	Maximal diastolic potential

Abbreviation	Definition
m.e.	Methyl ester
MenO	Menhaden oil
MEX	Mexiletine
Mg	Magnesium ATPase
MI	Myocardial infarction
MO	Mitochondrial oligomycin sensitive ATPase
MP	Metabolites and pathways
MUFA	Monounsaturated fatty acit
N	Non-government / non-profit
Na	Sodium
Na^+	Sodium
NA	Not available
Nad	Sodium dependent
Na/K	Sodium potassium
NB	No blocks
NC	No change
Na/H exch	Sodium/hydrogen exchanger
NCHS	National Center for Health Statistics
ND	No data
NDGA	Nordihydroguiarectic acid
NEMC	New England Medical Center
NEU	Neurokinin
NHANES III	National Health and Nutrition Examination 1988-1994
NIH	National Institutes of Health
NIT	Nitrendipine
NorEpi	Norepinephrine
NP	Not for Profit
O	Other (in evidence table)
O2	Oxygen
ODS	Office of Dietary Supplements
OO	Olive Oil
OS	overshoot potential
OUA	Ouabain
OvAI	ovalbumin
P	Prevented (when in footnote of table)
PAA	Phenylalkylamine
PAI	Plasminogen activator inhibitor
Pas	Passive
PCL	Percent cell length
PE/A	Pump efficiency or affinity for ATP
PG	Prostaglandin
PHE	Phenylephrine
PIR	Poverty Income Ratio
PLC-b	Receptor mediated phospholipase C
PPAR	Peroxisome proliferator activated receptor
PRP	Post rest potentiation
PTC	Palmitoylcarnitine
PUFA	Polyunsaturated fatty acid
QRS	Ventricular conductance time
Qt	Electrocardiogram interval
RCL	Resting cell length
RDA	Recommended Dietary Allowances
RDT	Resting/developed tension
ReOxy	Reoxygenation

Abbreviation	Definition
RO	Rapeseed oil (canola oil)
RP	Resting potential
RRP	Relative refractory period
RSE	Relative standard error
SAF	Safflower Oil
SC	Spontaneous contraction
SCL	Systolic cell length
SC-RAND	Southern California-RAND
SD	Standard deviation
SEM	Standard error of the mean
SF	Saturated fat
SFA	Saturated fatty acid
SL	Sarcolemma
SM3	Synthesized medium for omega-3 group
SM6	Synthesized medium for omega-6 group
SR	Sarcoplasmic reticulum
SREBP	Sterol regulatory element binding protein
STA or STD	Standard
SUP	Supplement
Sys	Systolic
T	Terminated (when in footnote of table)
TA	Twitch amplitude
TC	Total cholesterol
tC_{20}	Contracting coupling delay
TD	Time dependent
TEP	Technical Expert Panel
Tg	Triglycerides
TIC	Time constant of I_{to} inactivation
TIM	Timolol
TNF	Tumor necrosis factor
TPA	Tissue plasminogen activator
TS	Twitch size
TSR	Time in sinus rhythm
TT FA	Total Fatty Acids
Tx	Thromboxane
UO	University of Ottawa
USDA	United States Department of Agriculture
VCAM	Vascular cell adhesion molecule
VEB	Ventricular ectopic beats
VEN	Ventricular
VER	Verapamil
VERP	Left ventricular effective refractory period
VF	Ventricular fibrillation
VFT	Ventricular fibrillation threshold
VLDL	Very low density lipoprotein
VLN-3FA	Very long chain n-3 fatty acid
V_{max}	Maximum rate of depolarization
VP	Vasopressin
VPB	Ventricular premature beat
VS	Velocity of shortening
VSRM	Voltage-sensitive release mechanism
VT	Ventricular tachycardia
W/W	Weight-by-weight

Parameters

Arrhythmia-related parameters used in this report

Category	Sub Categories
Ion Channels, Pumps and Currents	• Basal Electromechanical Parameters - Resting potential (RP) - Action Potential Threshold (APT) - Action Potential Amplitude (APA) - Action Potential Duration at 40% repolarization (ADP_{40}) - Action Potential Duration at 80% repolarization (ADP_{80}) - Maximum rate of depolarization (V_{max}) - Maximum Diastolic Potential (MDP) - Overshoot potential or overshoot plateau potential (OS) • Ion Currents - Initial fast Na^+ current (I_{Na}) - Initial outward K^+ current/Transient K^+ outward current (I_{to}) - Voltage dependent L-type Ca^{2+} current/Inward Ca^{2+} current/Ca^{2+} sparks ($I_{Ca.L}$) - Delayed rectifier K^+ current (I_K) - Inward rectifier K^+ current (I_{KI}) or tail current - Caffeine and Neurokinin A elicited Ca^{2+} dependent Cl^- current ($I_{Cl.Ca}$) - Outward K^+ current (I_{SUS}) - Receptor mediated Ca^{2+} permeable non selective cation currents (?) - Kv4.3 current (?) • Ion Channels - Slow Ca^{2+} channel and L-type Ca^{2+} channel - Delayed rectifier K^+ channel - Kv1.1, Kv2.1and Kv1.5 channels - Na^+-K^+-$2Cl^-$ cotransporter • Ion Pumps and Ion Movement - Sodium Pump or Na, K-ATPase - Ca^{2+} influx or uptake or rise or cytosolic free Ca^{2+} - Na^+ dependent Ca^{2+} influx - Na^+/H^+ uptake - Passive Ca^{2+} efflux - Na^+ uptake - Sarcoplasmic reticulum (SR) Ca^{2+} content or release
Contractile Parameters	• Inotropic parameters (IP) - Frequency ($_F$) - Amplitude ($_A$) - Duration ($_D$) - Resting cell length ($_L$) • Arrhythmia (AR) • Action Potential Rate, or beating rate or frequency, or contraction rate (APR) • Contraction coupling delay (tC_{20}) • Contraction duration at 20% relaxation (CD_{20}) • Contraction duration at 80% relaxation (CD_{80}) • Relaxation time ($-C_{max}$) • Cell shortening velocity ($+C_{max}$)

Table 3-1. Summary of Study Design and Outcomes Evaluated in Whole Animal Studies (23 feeding and 3 infusion studies) *

Author, Year	Omega-3 Arm(s)	Control Arm*	Animals	Outcomes Evaluated							
				VF	VT	VPB	AS	Deaths	IS	TSR	VFT
Feeding studies:											
Omega-3 PUFAs vs Omega-6 PUFAs											
Abeywardena, 1995	Soybean, MaxEPA™	SSO	Rats	v	v	v	v	v			
Anderson, 1996	MaxEPA™	Safflower	Rats	v	v	v	v				
Charnock, 1992	Fish oil	SSO	Monkeys	v							v
Charnock, 1991	Fish oil	SSO	Rats	v	v	v	v				
Hock, 1990	Menhaden	Corn	Rats	v			v	v			
Hock, 1987	Menhaden	Corn	Rats			v		v			
Isensee, 1994	Linseed, Fish oil	Corn	Rats	v	v				v	v	
McLennan, 1995	Canola, Soybean	SSO	Rats	v	v	v	v	v			
McLennan, 1992	Tuna	SSO	Monkeys	v							v
McLennan, 1993	Fish oil	SSO	Rats	v	v	v	v	v		v	
McLennan, 1990	Tuna	SSO	Rats	v	v	v	v	v		v	
McLennan, 1988	Tuna	SSO	Rats	v	v		v	v	v		
McLennan, Bridle, 1993	Fish oil	SSO	Monkeys	v							v
Omega-3 PUFAs vs MUFAs											
McLennan, 1996	EPA-e, DHA-e, EPA-e+DHA-e	Olive	Rats	v			v				
Omega-3 PUFAs vs SFAs											
al Makdessi, 1995	Sardine	Coconut	Rats						v	v	
Chen, 1994	Fish oil	Coconut	Rabbits			v		v			
Hartog, 1987	Mackerel	Lard	Piglets	v	v	v		v			
Pepe, 1996	Fish oil	Sheep fat	Rats	v	v	v					v
Yang, 1993	Fish oil	Butter	Rats	v	v						
Omega-3 PUFAs vs Chows											
Culp, 1980	Menhaden	Friskies Dinner	Dogs			v		v	v		
Kinoshita, 1994	EPA-e	Oriental Yeast Co.	Dogs	v	v	v	v				
Oskarsson, 1993	MaxEPA™	Chows	Dogs						v		
Otsuji, 1993	EPA-e	Oriental Yeast Co.	Dogs					v	v	v	
	Total =			**17**	**12**	**12**	**10**	**11**	**6**	**5**	**4**
Infusion studies:											
Omega-3 PUFAs vs Omega-6 PUFAs											
Billman, 1999	Albumin-bound ALA, EPA, DHA	Soybean or saline	Dogs	v							
Billman, 1994	Fish oil emulsion	Soybean	Dogs	v							
Omega-3 PUFAs vs Chows											
Lo, 1991	ALA	Buffer	Dogs		v	v					
	Total =			**2**	**1**	**1**					

SSO = sunflower seed oil; VF=ventricular fibrillation; VT=ventricular tachycardia; VPB=ventricular premature beats; AS=arrhythmia score; IS=infarct size; VFT=ventricular fibrillation threshold, measured only in VF inducible animals; TSR =length of time in normal sinus rhythm; EPA-e = EPA esters; DHA-e = DHA esters

* For the purposes of our evidence review, only optimal comparison group was chosen. See Chapter 2: Methods.

Table 3-2. Summary Of Study Design And Outcomes Evaluated In Whole Animal/Isolated Organ And Cell Studies

Author	Species	Stage	Sex	Omega-3	Ctrl	Omega-3	Omega-3	ICU	ICH	IPIM	BEP	CP
Croset, 1989a	Mouse	W	M	STD+DHA-DOSE	STD					v		
Croset, 1989b	Mouse	W	M	ALAe	OO+SAF	EPAe	DHAe			v		
Benediktsdottir, 1988	Rat	A	M	CLO	CO					v		
Demaison, 1993	Rat	W	M	LIN	SF							v
Karmazyn, 1987	Rat	W	M+F	STD+CLO	STD					v	v	
Laustiola, 1986	Rat	W	M	STD+CLO	STD							v
Leifert, 2000	Rat	YA	M	FO	LARD			v				v
Minarovic, 1997	Rat	YA	M	FO	HF			v	v			
Taffet, 1993	Rat	YA	F	CO+MenO	CO					v		
Maixent, 1999	Rat	A	M	STD+FO	STD					v		
Chen, 1994	Rabbit	A	M	HC+FO	HC					v		
Heard, 1992	Rat	A	M	SAF+MenO	SAF							v
Gudmundsdottir, 1991	Rat	A,O	M	CLO	CO			v				
Reig, 1993	Rat	YA	M	HF+FO	HF						v	v
Ku, 1997	Rat	O	F	HC+EPA	HC	HC+DHA						v
Honen, 2002	Rat	A	M	FO	RO					v		
Leifert, 2001	Rat	A	M	FO	SF					v		v
Pepe, 1999	Rat	A,O	M	FO	N-6					v		
Swanson, 1989	Mouse	W	M	SAF+MenO	SAF+CO					v		
Gillis, 1992	Rabbit	W	ND	FO	SAF						v	
Kinoshita, 1994	Dog	A	ND	STD+EPAe	STF					v		
								3	**1**	**12**	**3**	**7**

ICU=ion currents; ICH=ion channels; IPIM=ion pumps and ion channels; BEP=basal electromechanical parameters; CP=contractile parameters.

A=Adult
ALAe= Esterified alpha linoleic acid
CLO=cod liver oil
CO=corn oil
EPAe= Esterified eicosapentaenoic acid
FO=fish oil
HC=high cholesterol
HF=high fat
LIN=linseed oil
MenO= menhaden oil
N-6=nOmega-6 fatty acid
O= old
OO=olive oil
RO= rapeseed or canola oil
SAF=safflower oil
SF=saturated fat
STD=standard chow
W= weanling
YA=Young adult

Table 3-3. Summary of Study Design and Outcomes Evaluated in Isolated Organ and Cell Studies

Author	Species	Stage*	ICU	ICH	IPIM	BEP	CP
Bogdanov, 1998	Rat	Adult	v			v	
Courtois, 1992	Rat	W					v
De Jonge, 1996	Rat	W					v
Hallaq, 1990	Rat	W			v		v
Hallaq, 1992	Rat	W		v	v		v
Honore, 1994	Mouse	W	v	v			
Jahangiri, 2000	Rat	Adult					v
Kang, 1994	Rat	W					v
Juan, 1987	Guinea pig	Adult					v
Xiao, 2002	Ferret	Adult	v				
Kang, 1996	Rat	W			v		v
Leifert, 1999	Rat	Adult	v				
Leifert, 2000	Rat	Adult					v
Rodrigo, 1999	Rat, guinea pig	ND	v				v
MacLeod, 1998	Rat, guinea pig	Adult	v				v
O'Neill, 2002	Rat	Not sure	v		v		
Durot, 1997	Rat	W				v	v
Grynberg, 1988	Rat	W				v	v
Kang, 1995a	Rat	W				v	
Kang, 1997	Rat	W		v			
Li, 1997	Rat	W					v
Negretti, 2000	Rat	ND	v		v		v
Pepe, 1994	Rat	2-3 mo	v		v		v
Phillipson, 1985	Dog	ND			v		
Phillipson, 1987	Dog	ND			v		
Grynberg, 1996	Rat	W				v	v
Kang, 1995b	Rat	W					v
Fournier, 1995	Rat	W				v	v
Grynberg, 1995	Rat	W					v
Ferrier, 2002	Guinea pig	Adult	v				v
Reithman, 1996	Rats	W				v	v
Ponsard, 1999	Rats	W					v
Xiao, 1997	Rats	Adult	v		v		
Xiao, 1995	Rats	W	v				
Goel, 2002	Pig	Adult			v		
Vitelli, 2002	Rats	Adult			v		
Weylandt,1996	Rats	W				v	
Rinaldi, 2002	Rats	Adult			v	v	
Bayer, 1979	Cat	Adult				v	
Total			12	3	12	10	23

ICU=ion currents; ICH=ion channels; IPIM=ion pumps and ion channel movement ; BEP=basal electromechanical parameters; CP=contractile parameters.

*Stage: ND=no data; W = weanling

Table 3-4. Total Deaths in Ischemia-Reperfusion-Induced Arrhythmia: Comparison of Rats Fed Omega-3 Fatty Acids With Controls Fed Omega-6 PUFA Oils

Author, Year	Omega-3 Arms	Dosage, g/100 g	Duration	Omega-3 Fatty Acids		Control		RR (95% CI)	Experiment Protocols
				Event	Total	Event	Total		
ALA Oils									
Abeywardena, 1995	Soybean	0.4	9 months	2	18	1	18	2.0 (1.5-20)	5-min ischemia; 10-min reperfusion
McLennan, 1995	Soybean	1.1	5 weeks	3	10	2	10	1.5 (0.32-7.1)	5-min ischemia; reperfusion
								1.3 (0.25-6.8)	
McLennan, 1995	Soybean	1.1	5 weeks	2 *	13	2 †	14	0.20 (0.01-3.7)	15-min ischemia; reperfusion
								1.3 (0.25-6.8)	
McLennan, 1995	Canola	1.2	5 weeks	0	10	2	10	0.20 (0.01-3.7)	5-min ischemia; reperfusion
McLennan, 1995	Canola	1.2	5 weeks	3 ‡	16	2 †	14	1.1 (0.18-6.6)	15-min ischemia; reperfusion
Meta-analysis: Total subjects = 133				**10**	**67**	**9**	**66**	**1.2 (0.51-2.6)**	**Random-effect model**
Fish Oils (EPA+DHA)									
Hock, 1987	Menhaden	1.0	4 weeks	2 ‡	13	2 ‡	14	1.1 (0.18-6.6)	15-min after ischemia without reperfusion
Hock, 1990	Menhaden	1.0	4 weeks	5	21	13	22	0.40 (0.17-0.93)	15-min ischemia; 24 h reperfusion
McLennan, 1993	Fish oil	2.6	12 weeks	0	10	1 *	12	0.39 (0.02-8.7)	5-min ischemia; 5-min reperfusion
McLennan, 1993	Fish oil	2.6	12 weeks	0	14	1 *	13	0.31 (0.01-7.0)	15-min ischemia; 5-min reperfusion
Abeywardena, 1995	MaxEPA™	3.3	9 months	0	18	1	18	0.33 (0.01-7.7)	5-min ischemia; 10-min reperfusion
McLennan, 1988	Tuna	3.7	12 months	0	10	0	10	1.0 (0.02-46)	15-min ischemia; reperfusion
McLennan, 1990	Tuna	3.7	18 months	0	7	0	7	1.0 (0.02-45)	15-min ischemia; reperfusion
Meta-analysis: Total subjects = 169				**7**	**83**	**18**	**86**	**0.47 (0.23-0.93)**	**Random-effect model**

RR = risk ratio = (omega-3 FA event rate)/(control's event rate)

* All deaths occurred during ischemia procedure

† One death occurred during ischemia procedure

‡ Deaths were observed 15-min after ischemia procedure without reperfusion

Figure 3-1. Total deaths in ischemia-reperfusion-induced arrhythmia: comparison of rats fed, alpha linolenic acid (ALA) with controls fed omega-6 PUFA oils

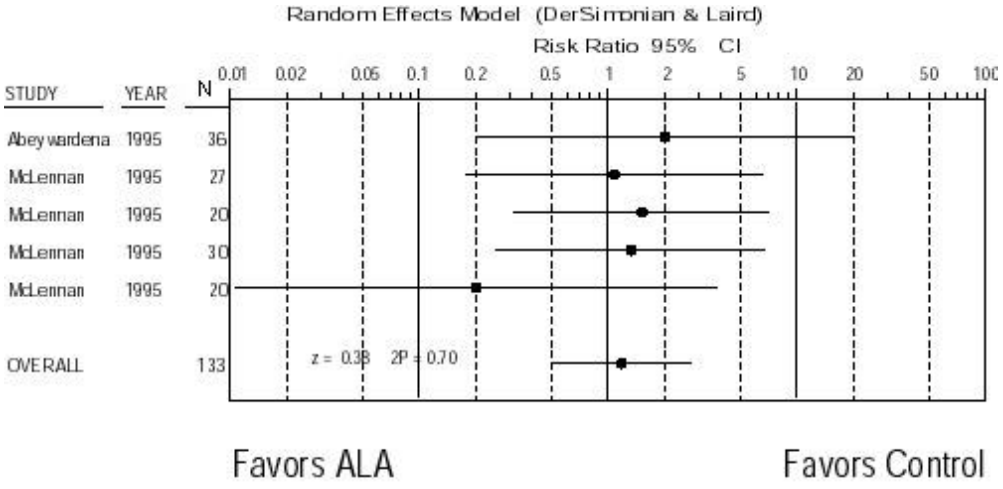

Figure 3-2. Total deaths in ischemia-reperfusion-induced arrhythmia: comparison of rats fed fish oils with controls fed omega-6 PUFA oils

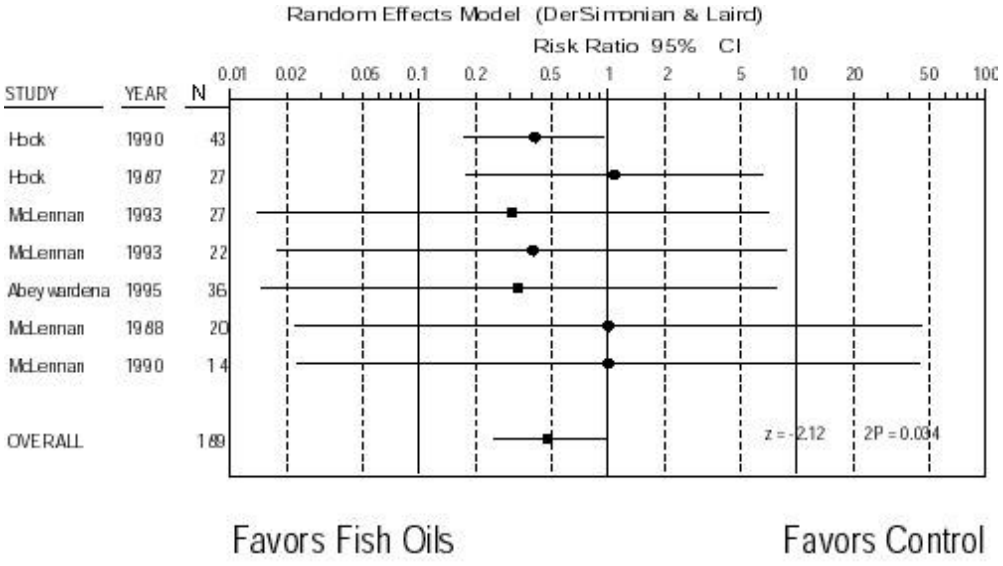

Table 3-5. Sensitivity Analysis on Total Deaths In Ischemia-ReperfusionInduced Arrhythmia: Comparison of Rats Fed Fish Oil With Controls Fed Omega-6 PUFA Oils

Sensitivity Analysis - Sequential Dropping of One Study
Random Effects Model - Risk Ratio (D&L method)

Study Dropped	Study Year	Size	Total N	Risk Ratio	95% CI Low	95% CI High	2P
Hock	1987	27	142	0.41	0.19	0.86	0.018
Hock	1990	43	126	0.64	0.19	2.14	0.47
McLennan	1993	22	147	0.47	0.23	0.96	0.038
McLennan	1993	27	142	0.48	0.24	0.97	0.041
Abeywardena	1995	36	133	0.48	0.23	0.97	0.040
McLennan	1990	14	155	0.46	0.23	0.92	0.028

Table 3-6. Total VF Deaths: Comparison of Monkeys Fed Tuna Fish Oil With Controls Fed Sunflower Seed Oil (Omega-6 PUFA) *

Author, Year	Omega-3 Arms	Dosage, g/100 g	Duration	Omega-3 Fatty Acids		Control		Experiment Protocols
				Event	Total	Event	Total	
McLennan, 1992	Tuna	2.8	30 months	0	16	3	13	Control condition, ischemia, and isoproterenol (0.5 ug/kg body weight/minute) models

* Total ventricular fibrillation (VF) deaths were combined in control condition, ischemia, and isoproterenol models.

Table 3-7. Ventricular Tachycardia in Ischemia-Induced Arrhythmias: Comparison of Rats Fed Omega-3 Fatty Acids With Controls Fed Omega-6 PUFA Oils

Author, Year	Omega-3 Arms	Dosage, g/100 g	Duration	Omega-3 Fatty Acids		Control		RR (95% CI)	Experiment Protocols
				Event	Total	Event	Total		
ALA Oils									
Abeywardena, 1995	Soybean	0.4	9 months	8	18	7	18	1.1 (0.53-2.5)	5-min ischemia
McLennan, 1995	Soybean	1.1	12 weeks	8	13	13	14	0.66 (0.42-1.0)	15-min ischemia
McLennan, 1995	Canola	1.2	12 weeks	12	16	13	14	0.81 (0.591.1)	15-min ischemia
Isensee, 1994	Linseed	5.2	10 weeks	6	10	4	9	1.4 (0.56-3.3)	20-min ischemia
	Meta-analysis:	**Total subjects = 112**		**34**	**57**	**37**	**55**	**0.82 (0.65-1.0)**	**Random-effect model**
Fish Oils (EPA+DHA)									
Charnock, 1991	Fish oil	2.1	12 months	7	10	10	10	0. 71 (0.41-1.1)	15-min ischemia
McLennan, 1993	Fish oil	2.6	12 weeks	5	14	12	13	0.39 (0.19-0.79)	15-min ischemia
Isensee, 1994	Fish oil	3.0	10 weeks	0	10	4	9	0.10 (0.01-1.7)	20-min ischemia
Abeywardena, 1995	MaxEPA	3.3	9 months	1	18	7	18	0.14 (0.02-1.1)	5-min ischemia
McLennan, 1988	Tuna	3.7	12 months	2	10	8	10	0.25 (0.07-0.90)	15-min ischemia
McLennan, 1990	Tuna	3.7	18 months	4	7	4	7	1.0 (0.40-2.5)	15-min ischemia
	Meta-analysis:	**Total subjects = 136**		**19**	**69**	**45**	**67**	**0.49 (0.29-0.83)**	**Random-effect model**

Figure 3-3. Ventricular tachycardia in ischemia-induced arrhythmias: comparison of rats fed alpha linolenic acid (ALA) with controls fed omega-6 PUFA oils

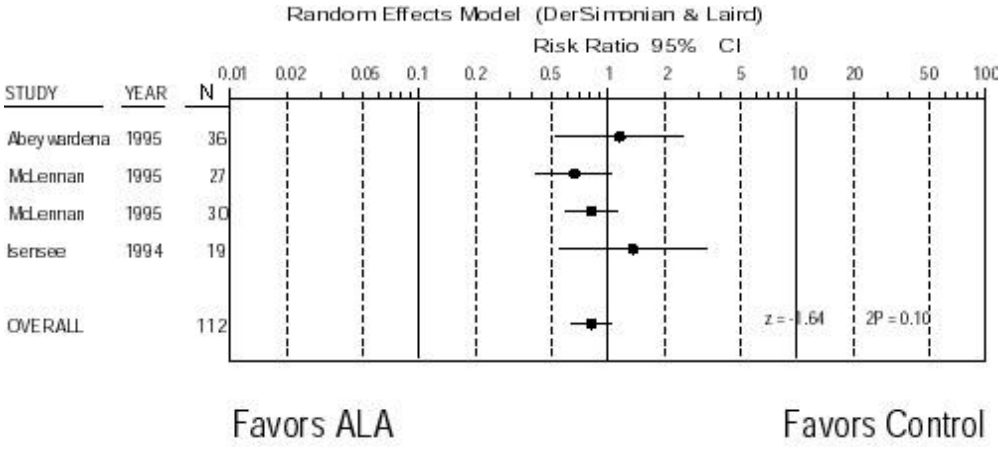

Figure 3-4. Ventricular tachycardia in ischemia-induced arrhythmias: comparison of rats fed fish oils with controls fed omega-6 PUFA oils

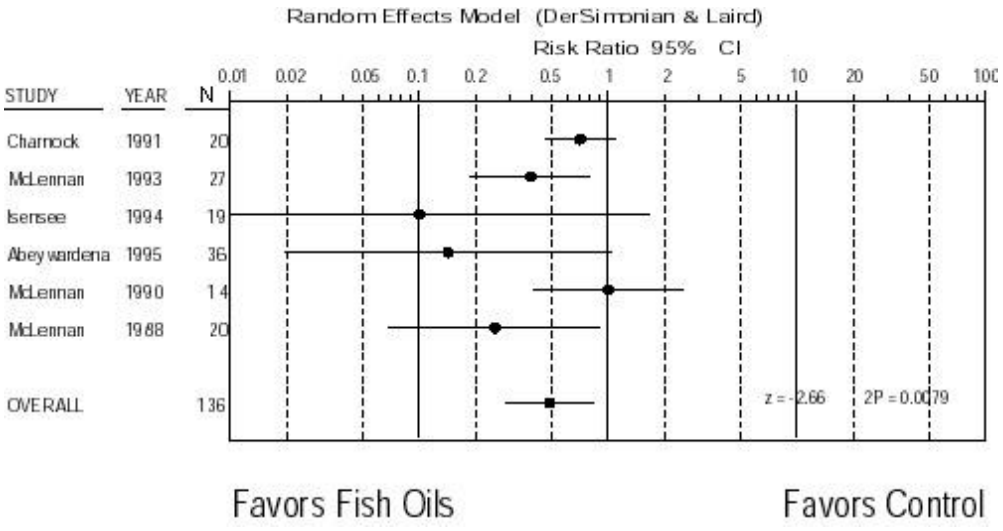

Table 3-8. Ventricular Tachycardia in Reperfusion-Induced Arrhythmias: Comparison of Rats Fed Omega-3 Fatty Acids With Controls Fed Omega-6 PUFA Oils

Author, year	Omega-3 Arms	Dosage, g/100 g	Duration	Omega-3 Fatty Acids		Control		RR (95% CI)	Experiment Protocols
				Event	Total	Event	Total		
ALA oils									
Abeywardena, 1995	Soybean	0.4	9 months	13	17	7	18	2.0 (1.0-3.7)	5-min Ischemia; 10-min Reperfusion
McLennan, 1995	Soybean	1.1	12 weeks	9	10	7	10	1.3 (0.82-2.0)	5-min Ischemia; 10-min Reperfusion
McLennan, 1995	Soybean	1.1	12 weeks	7	11	9	13	0.92 (0.52-1.6)	15-min Ischemia; 10-min Reperfusion
McLennan, 1995	Canola	1.2	12 weeks	7	10	7	10	1.0 (0.56-1.8)	5-min Ischemia; 10-min Reperfusion
McLennan, 1995	Canola	1.2	12 weeks	4	13	9	13	0.44 (0.18-1.1)	15-min Ischemia; 10-min Reperfusion
	Meta-analysis:	**Total subjects = 125**		**40**	**61**	**39**	**64**	**1.1 (0.73-1.6)**	**Random-effect model**
Fish Oils (EPA+DHA)									
Anderson, 1996	MaxEPA	41% of TT FAs	8 weeks	3 *	8	3 *	6	0.75 (0.23-2.5)	20-min ischemia; reperfusion
McLennan, 1993	Fish oil	2.6	12 weeks	6	10	10	12	0.72 (0.41-1.3)	5-min Ischemia; 5-min Reperfusion
McLennan, 1993	Fish oil	2.6	12 weeks	3	14	8	12	0.32 (0.11-1.0)	15-min ischemia; 5-min reperfusion
Abeywardena, 1995	MaxEPA	3.3	9 months	4	18	7	18	0.57 (0.20-1.6)	5-min ischemia; 10-min reperfusion
McLennan, 1988	Tuna	3.7	12 months	5	10	8	10	0.63 (0.31-1.3)	15-min ischemia; reperfusion
McLennan, 1990	Tuna	3.7	18 months	5	7	6	7	0.83 (0.48-1.5)	15-min ischemia; 10-min reperfusion
	Meta-analysis:	**Total subjects = 132**		**26**	**67**	**42**	**65**	**0.68 (0.50-0.91)**	**Random-effect model**

TT FAs = total fatty acids; RR = risk ratio; VF = ventricular fibrillation; VT = ventricular tachycardia

* Sustained VT and/or VF were excluded from the analyses

Figure 3-5. Ventricular tachycardia in reperfusion-induced arrhythmias: comparison of rats fed alpha linolenic acid (ALA) with controls fed omega-6 PUFA oils

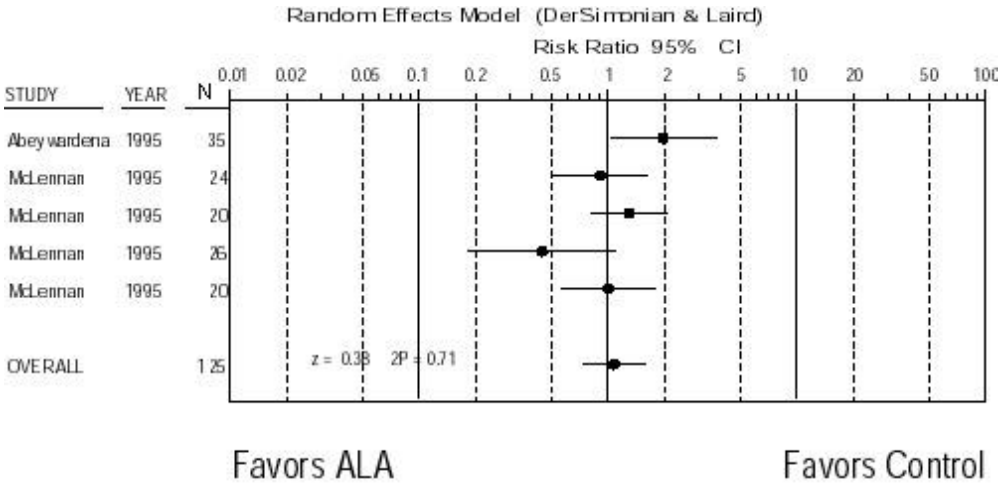

Figure 3-6. Ventricular tachycardia in reperfusion-induced arrhythmias: comparison of rats fed fish oils with controls fed omega-6 PUFA oils

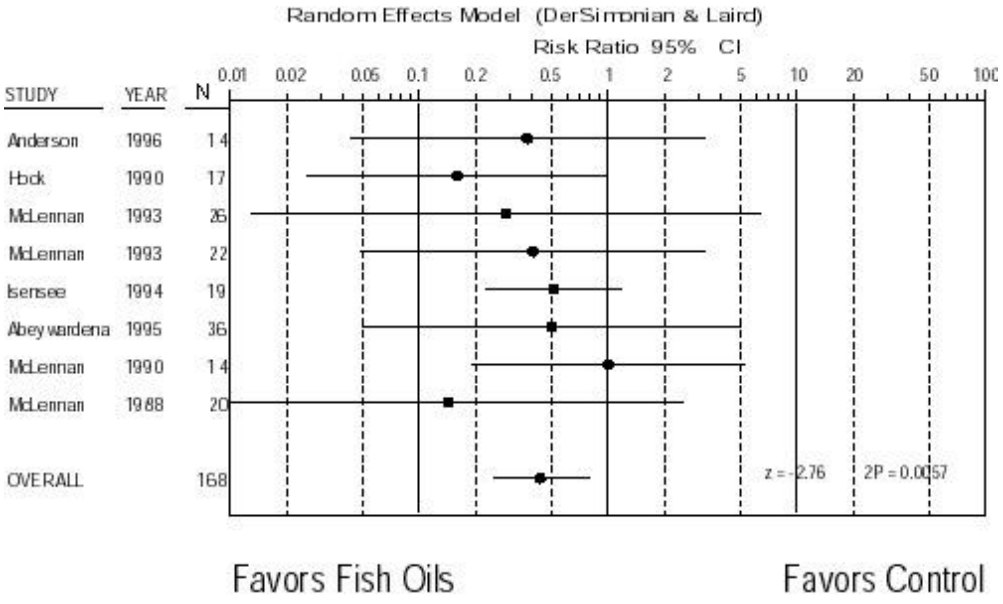

Table 3-9. Ventricular Fibrillation in Ischemia-Induced Arrhythmias: Comparison of Rats Fed Omega-3 Fatty Acids With Controls Fed Omega-6 PUFA Oils

Author, Year	Omega-3 Arms	Dosage, g/100 g	Duration	Omega-3 Fatty Acids		Control		RR (95% CI)	Experiment Protocols
				Event	Total	Event	Total		
ALA oils									
McLennan, 1995	Soybean	1.1	12 weeks	5	13	6	14	0.90 (0.36-2.2)	15-min ischemia
McLennan, 1995	Canola	1.2	12 weeks	7	16	6	14	1.0 (0.45-2.3)	15-min ischemia
Isensee, 1994	Linseed	5.2	10 weeks	4	10	4	9	0.90 (0.31-2.6)	20-min ischemia
	Meta-analysis: Total subjects = 76			**16**	**39**	**16**	**37**	**0.95 (0.56-1.6)**	**Random-effect model**
Fish Oils (EPA+DHA)									
Charnock, 1991	Fish oil	2.1	12 months	0	10	6	10	0. 08 (0.00-1.2)	15-min ischemia
McLennan, 1993	Fish oil	2.6	12 weeks	0	14	5	13	0.08 (0.01-1.4)	15-min ischemia
Isensee, 1994	Fish oil	3.0	10 weeks	1	10	4	9	0.22 (0.03-1.7)	20-min ischemia
McLennan, 1988	Tuna	3.7	12 months	0	10	1*	10	0.33 (0.02-7.3)	15-min ischemia
McLennan, 1990	Tuna	3.7	18 months	1	7	2	7	0.50 (0.06-4.3)	15-min ischemia
	Meta-analysis: Total subjects = 100			**2**	**51**	**18**	**49**	**0.21 (0.07-0.63)**	**Random-effect model**

* Estimated from graph

Figure 3-7. Ventricular fibrillation in ischemia-induced arrhythmias: comparison of rats fed alpha linolenic acid (ALA) with controls fed omega-6 PUFA oils

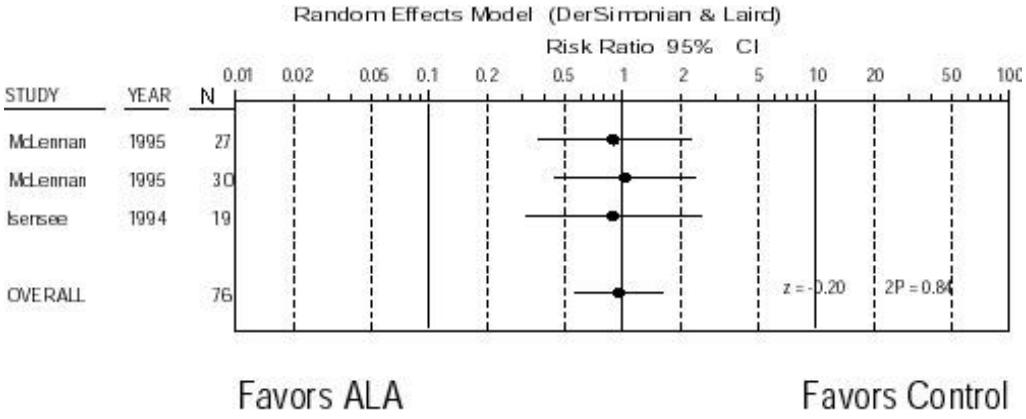

Figure 3-8. Ventricular fibrillation in ischemia-induced arrhythmias: comparison of rats fed fish oils with controls fed omega-6 PUFA oils

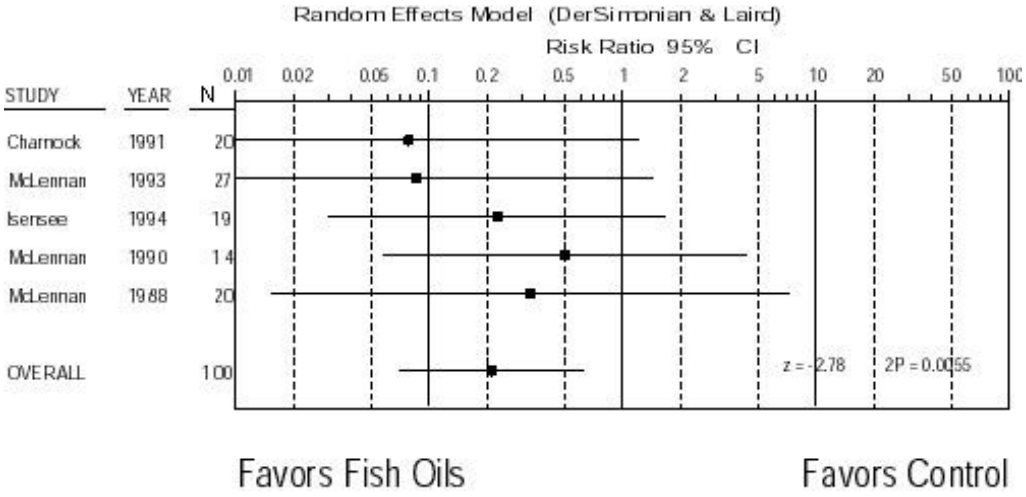

Table 3-10. Ventricular Fibrillation in Reperfusion-Induced Arrhythmias: Comparison of Rats Fed Omega-3 Fatty Acids With Controls Fed Omega-6 PUFA Oils

Author, Year	Omega-3 Arms	Dosage, g/100 g	Duration	Omega-3 Fatty Acids		Control		RR (95% CI)	Experiment Protocols
				Event	Total	Event	Total		
ALA Oils									
Abeywardena, 1995	Soybean	0.4	9 months	4	17	2	18	2.1 (0.44-10)	5-min ischemia; 10-min reperfusion
McLennan, 1995	Soybean	1.1	12 weeks	5	10	5	10	1.0 (0.42-2.4)	5-min Ischemia; Reperfusion
McLennan, 1995	Soybean	1.1	12 weeks	3	11	3	13	1.2 (0.30-4.7)	15-min ischemia; reperfusion
McLennan, 1995	Canola	1.2	12 weeks	1	10	5	10	0.20 (0.03-1.4)	5-min ischemia; reperfusion
McLennan, 1995	Canola	1.2	12 weeks	0	13	3	13	0.14 (0.01-2.5)	15-min ischemia; reperfusion
Isensee, 1994	Linseed	5.2	10 weeks	6	10	7	9	0.77 (0.42-1.4)	20-min ischemia; 20-min reperfusion
	Meta-analysis:	**Total subjects = 144**		**19**	**71**	**25**	**73**	**0.84 (0.52-1.3)**	**Random-effect model**
Fish Oils (EPA+DHA)									
Anderson, 1996	MaxEPA™	41% of TT FAs	8 weeks	1 *	8	2 *	6	0.38 (0.04-3.2)	20-min ischemia; reperfusion
Hock, 1990	Menhaden	1.2	4 weeks	1 †	7	9 †	10	0.16 (0.03-0.99)	15-min ischemia; 6-hr reperfusion
McLennan, 1993	Fish oil	2.6	12 weeks	1	10	3	12	0.40 (0.05-3.3)	5-min ischemia; 5-min reperfusion
McLennan, 1993	Fish oil	2.6	12 weeks	0	14	1	12	0.29 (0.01-6.5)	15-min ischemia; 5-min reperfusion
Isensee, 1994	Fish oil	3.0	10 weeks	4	10	7	9	0.51 (0.22-1.2)	20-min ischemia; 20-min reperfusion
Abeywardena, 1995	MaxEPA™	3.3	9 months	1	18	2	18	0.50 (0.05-5.0)	5-min ischemia; 10-min reperfusion
McLennan, 1988	Tuna	3.7	12 months	0	10	3	10	0.14 (0.01-2.5)	15-min ischemia; reperfusion
McLennan, 1990	Tuna	3.7	18 months	2	7	2	7	1.0 (0.19-5.2)	15-min ischemia; reperfusion
	Meta-analysis:	**Total subjects = 168**		**10**	**84**	**29**	**84**	**0.44 (0.25-0.79)**	**Random-effect model**

TT FA = total fatty acids; VT = ventricular tachycardia; VF = ventricular fibrillation
* Sustained VT and/or VF were excluded from the analyses
† VT or VF (%)

Figure 3-9. Ventricular fibrillation in reperfusion-induced arrhythmias: comparison of rats fed alpha linolenic acid (ALA) with controls fed omega-6 PUFA oils

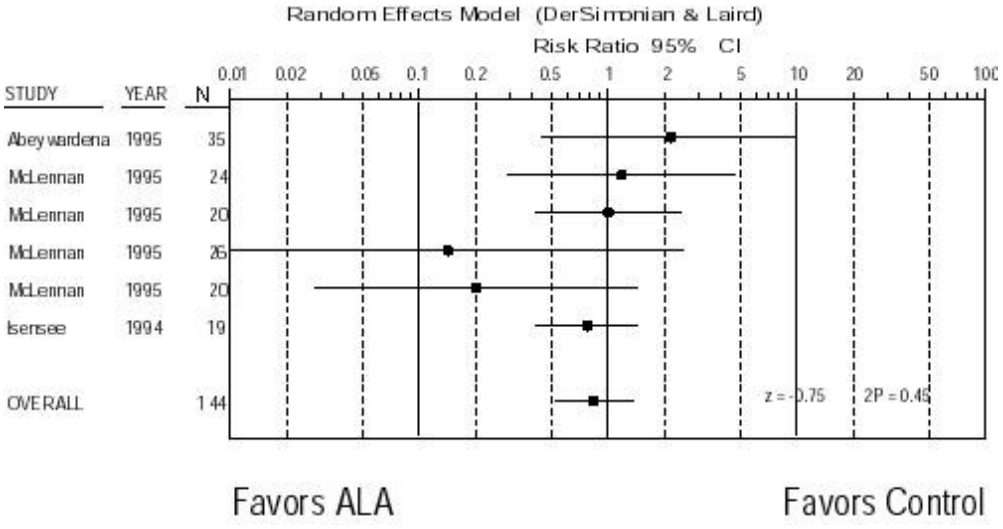

Figure 3-10. Ventricular fibrillation in reperfusion-induced arrhythmias: comparison of rats fed fish oils with controls fed omega-6 PUFA oils

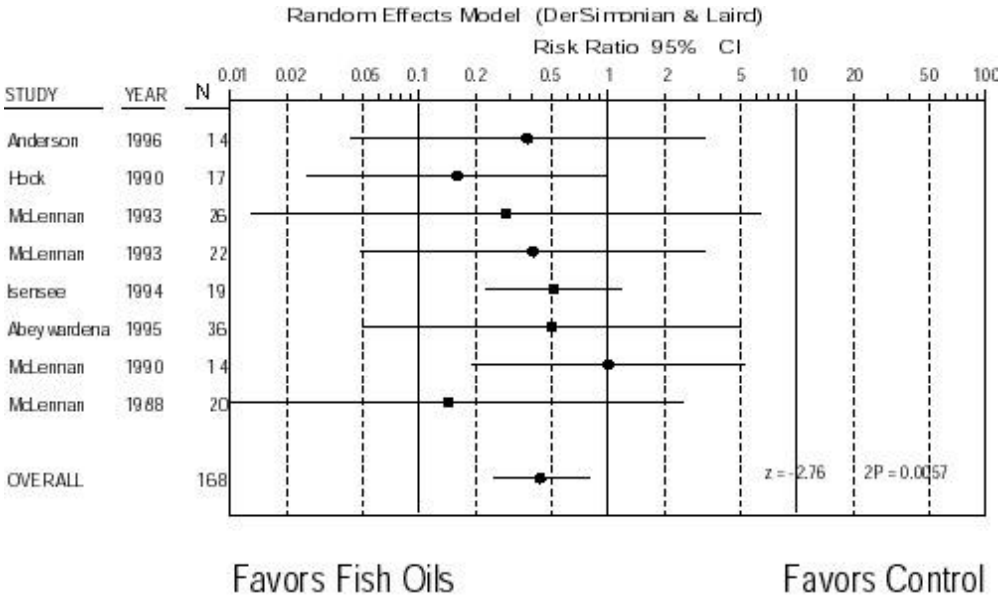

Table 3-11. Ventricular Fibrillation in Induced Arrhythmia: Comparison of Monkeys Fed Fish Oils With Controls Fed Sunflower Seed Oil (Omega-6 PUFA)

Author, Year	Omega-3 Arms	Dosage, g/100 g	Duration	Omega-3 Fatty Acids		Control		VFT ¶	Experiment Protocols
				Event	Total	Event	Total		
Electrical-Stimulation Arrhythmias †									
McLennan, Bridle, 1993	Fish oil	1.8	16 weeks	6	10	5	9	+133% *	Electrical stimulation in control condition
Charnock, 1992	Fish oil	2.4	16 weeks	8%	ND	13%	ND	NS	Electrical stimulation in control condition
McLennan, 1992	Tuna	2.8	30 months	10	16	8	13	NS	Electrical stimulation in control condition
Electrical-Stimulation Arrhythmias in Ischemic Hearts †									
McLennan, Bridle, 1993	Fish oil	1.8	16 weeks	10	10	9	9	+79% *	Electrical stimulation + 5-min ischemia
Charnock, 1992	Fish oil	2.4	16 weeks	Nil	ND	13%	ND	NS	Electrical stimulation + ischemia
McLennan, 1992	Tuna	2.8	30 months	12	16	8	13	NS	Electrical stimulation + 5-min ischemia
Electrical-Stimulation Arrhythmias With Isoproterenol †									
McLennan, Bridle, 1993	Fish oil	1.8	16 weeks	3	10 ‡	7	9 ‡	+55% *	Electrical stimulation + 30-min isoproterenol (0.5 ug/kg BW/min)
McLennan, Bridle, 1993	Fish oil	1.8	16 weeks	5	10 ‡	9	9 ‡	+75%	Electrical stimulation + 30-min isoproterenol (2.0 ug/kg BW/min)
McLennan, 1992	Tuna	2.8	30 months	7	16	10	13	NS	Electrical stimulation + 30-min Iisoproterenol (0.5 ug/kg BW/min)

ND = no data; BW = body weight; min = minute; VFT = ventricular fibrillation threshold, measured only in VF inducible animals; NS = no significant difference compared to controls

* $P<0.05$ compared to control animals

† Same monkeys underwent electrical stimulation in control condition, 5 minutes after ischemia. procedure, and 30 minutes after restoration of coronary blood flow during the infusion of isoproterenol.

‡ Same monkeys injected 0.5 ug/kg BW/min isoproterenol, then the dosage of isoproterenol was increased to 2.0 ug/kg BW/min.

¶ An increase in VFT is a desirable outcome for antiarrhythimic effects. See Chapter 2: Methods for the effects expressed as percent change.

Table 3-12. Ventricular Premature Beats/Complex, Infarct Size, Arrhythmia Score and Length of Time in Normal Sinus Rhythm: Comparison of Rats Fed Omega-3 Fatty Acids With Controls Fed Omega-6 PUFA Oils

Author, Year	Omega-3 Arms	Dosage, g/100 g	Duration	Total N	Arrhythmia Outcomes [1]				Experimental Protocols
					VPB	AS [2]	IS	TSR [3]	
ALA Oils									
Abeywardena, 1995	Soybean	0.4	9 months	36	+176%	+107%*	-	-	5-min ischemia; 10-min reperfusion
McLennan, 1995	Canola	1.1	5 weeks	30	-13%	-11%	-	-	15-min ischemia
					-43%	-64%	-	-	10-min reperfusion
McLennan, 1995	Canola	1.1	5 weeks	20	-19%	–41%*	-	-	5-min ischemia; 10-min reperfusion
Isensee, 1994	Linseed	5.2	10 weeks	20	-	-	NS	NS	20-min ischemia
McLennan, 1995	Soybean	1.2	5 weeks	27	-14%	-18%	-	-	15-min ischemia
					-2%	-12%	-	-	10-min reperfusion
McLennan, 1995	Soybean	1.2	5 weeks	20	+34%	+30%	-	-	5-min ischemia; 10-min reperfusion
Fish Oils (EPA+DHA)									
Anderson, 1996	MaxEPA	41% of TT FAs	8 weeks	14	-31%	-54%	-	-	20-min ischemia; reperfusion
Hock, 1990	Menhaden	1.0	4 weeks	17	-	-77% †	-	-	15-min ischemia; 6-hr reperfusion
Hock, 1987	Menhaden	1.0	4 weeks	23	NC	-	-	-	15-min after ischemia w/o reperfusion
Charnock, 1991	Fish oil	2.1	12 months	20	-72%*	-59%*	-	-	15-min ischemia
McLennan, 1993	Fish oil	2.6	12 weeks	27	-10%	-41%*	-	+12%	15-min ischemia
					-31%	-63%*	-	+2%	5-min reperfusion
McLennan, 1993	Fish oil	2.6	12 weeks	22	-27%	-48%	-	+16%	5-min ischemia; 5-min reperfusion
Isensee, 1994	Fish oil	3.0	10 weeks	20	-	-	NS	Increased*	20-min ischemia
Abeywardena, 1995	MaxEPA	3.3	9 months	36	-13%	-40%	-	-	5-min ischemia; 10-min reperfusion
McLennan, 1990	Tuna	3.7	18 months	14	+6%	NS	-	-5%	15-min ischemia
					-24%*	NS		+21%	10-min reperfusion
McLennan, 1988	Tuna	3.7	12 months	20	-	NS	+7%, NS	-	15-min ischemia reperfusion

TT FAs = total fatty acids; VPB = ventricular premature beats/complex; IS = infarct size/size of ischemia zone; AS = arrhythmia score (according to Curtis et. al.); TSR =length of time in normal sinus rhythm; ISO = Isoproterenol

- Not reported NS = no significant difference compared to controls

* $P<0.05$ compared to controls † $P<0.01$ compared to controls

[1] See Methods for the effects expressed as percent change.

[2] AS in all studies were calculated according to Curtis et al [Cardiovascular Research 22, 656-665], except Hock, 1990 used a modified method

[3] An increase in TSR is a desirable outcomes for antiarrhythmic effects.

Table 3-13. Arrhythmic Effects in Studies Comparing Omega-3 Long-Chain PUFAs with a Linolenic Acid

Author, Year	Omega-3 Arms	Dosage, g/100 g	Duration	Animal	Sample Size	Arrhythmia Outcomes							Experimental Protocols
						Deaths	VT	VF	VPB /10 min	IS	AS	TSR ¶, min	
Abeywardena, 1995	Soybean	0.4	9 months	Rats	18	11%	76%	23%	298	-	3.1	-	5-min ischemia
	MaxEPA	3.3	9 months	Rats	18	0%	22%	5%	94	-	0.9	-	10-min reperfusion
Isensee, 1994	Linseed	5.2	10 weeks	Rats	10	-	60% -	40% 60%	-	35%* -	-	5.5*† -	20-min ischemia 20-min reperfusion
	Fish oil	3.0	10 weeks	Rats	10	-	0% -	10% 40%	-	36%* -	-	10*† -	20-min ischemia 20-min reperfusion

VT = ventricular tachycardia; VF = ventricular fibrillation; VPB = ventricular premature beats; IS = infarct size/size of ischemia zone;
AS = arrhythmia score (according to Curtis et. al.); TSR = length of time in normal sinus rhythm; min = minute
- Not reported * estimated value from figures † $p<0.05$ between groups
¶ An increase in TSR is a desirable outcomes for antiarrhythmic effects

Table 3-14. Total Deaths in Ischemia-Reperfusion-Induced Arrhythmias: Comparison of Animals Fed Fish Oil (EPA+DHA) With Controls Fed Saturated Fats

Author, year	Omega-3 Arms	Dosage, g/100 g	Duration	Omega-3 Fatty Acids		Control		Experiment Protocols
				Event	Total	Event	Total	
Rabbits								
Chen, 1994	Fish oil	5.2 %kcal	2 weeks	3 *	12	5 *	14	10-min ischemia; 1-hr reperfusion
Chen, 1994	Fish oil	5.2 %kcal	2 weeks	6 †	14	8 †	15	1-hr ischemia; 4-hr reperfusion
Piglets								
Hartog, 1987	Mackerel	0.6	16 weeks	1	7	0	6	5-min ischemia; 10-min reperfusion

* Two deaths in each group occurred during reperfusion
† 50% deaths occurred during ischemia; 50% occurred during reperfusion

Table 3-15. Ventricular Tachycardia in Ischemia-Reperfusion-Induced Arrhythmias: Comparison of Animals Fed Fish Oil With Controls Fed Saturated Fats

Author, Year	Omega-3 Arms	Dosage, g/100 g	Duration	Omega-3 Fatty Acids		Control		Experiment Protocols
				Event	Total	Event	Total	
Rats								
Pepe, 1996	Fish oil	5.2	16 weeks	7 †	20	14 †	20	15-min ischemia; 10-min reperfusion
Piglets								
Hartog, 1987	Mackerel	0.6	16 weeks	2 *	7	1 *	6	5-min ischemia; 10-min reperfusion

[1] All events occurred during ischemia procedure
† Some events occurred during ischemia; some occurred during reperfusion

Table 3-16. Ventricular Fibrillation in Ischemia-Reperfusion-Induced Arrhythmias: Comparison of Animals Fed Fish Oil With Controls Fed Saturated Fats

Author, Year	Omega-3 Arms	Dosage, g/100 g	Duration	Omega-3 Fatty Acids		Control		Experiment Protocols
				Event	Total	Event	Total	
Rats								
Pepe, 1996	Fish oil	5.2 %kcal	16 weeks	0	20	16	20	15-min ischemia; 10-min reperfusion
Yang, 1993	Fish oil	5.4 %kcal	5 days	3 *	8	7 *	9	15-min ischemia; 10-min reperfusion
Piglets								
Hartog, 1987	Mackerel	0.6	16 weeks	3 †	7	0	6	5-min ischemia; 10-min reperfusion

* VT (%) or VF (%). All events occurred during reperfusion
† Some events occurred during ischemia; some occurred during reperfusion

Table 3-17. Ventricular Premature Beats in Ischemia-Reperfusion-Induced Arrhythmias: Comparison of Animals Fed Fish Oil With Controls Fed Saturated Fats

Author, Year	Omega-3 Arms	Dosage, g/100 g	Duration	Animals	Total N	VPB [1]	Experiment Protocols
Ischemia-Induced Arrhythimas							
Chen, 1994 [2]	Fish oil	5.2 %kcal	2 weeks	Rabbits	22	-50% -35%	10-min ischemia 1-hr ischemia
Hartog, 1987	Mackerel	0.6	16 weeks	Piglets	13	+53%	5-min ischemia
Pepe, 1996	Fish oil	5.2 %kcal	5 days	Rats	40	-73%*	15-min ischemia
Reperfusion-Induced Arrhythimas							
Chen, 1994 [2]	Fish oil	5.2 %kcal	2 weeks	Rabbits	22	0% -25%	10-min ischemia; 1-hr reperfusion 1-hr ischemia; 4-hr reperfusion
Hartog, 1987	Mackerel	0.6	16 weeks	Piglets	13	-65%*	15-min ischemia; 10-min reperfusion

VPB = ventricular premature beat
*P<0.05

[1] See Chapter 2: Methods for the effects expressed as percent change
[2] Study results were biased by excluding more subjects who died from arrhythmias in the control group

Table 3-18. Total Deaths in Induced Arrhythmias: Comparison of Dogs Fed EPA and/or DHA With No Treatment Controls

Author, Year	Omega-3 Arms	Dosage, g/100 g	Duration	Omega-3 Fatty Acids		Control		Experiment Protocols
				Event	Total	Event	Total	
Culp, 1980	Menhaden	3.3 %kcal	5~7 weeks	3	10	5	17	Coronary artery thrombosis induced by electrical stimulation
Otsuji, 1993	EPA ester	1.0	8 weeks	0	10	5	15	Coronary artery ligation (or ischemia)

Table 3-19. Ventricular Premature Beats/Complex, Infarct Size, Arrhythmia Score and Areas at Risk of Arrhythmias: Comparison of Dogs Fed EPA and/or DHA With No Treatment Controls

Author, Year	Omega-3 Arms	Dosage, g/100 g	Duration	Total N	Arrhythima Outcomes [1]				Experimental Protocols
					VPB	AS [3]	IS	ARAr	
Kinoshita, 1994	EPA ester	1.0	8 weeks	20	-44%*	-55% †	-	-	3-hr ischemia
Culp, 1980	Menhaden	3.3 %kcal	5~7 weeks	27	Decreased	-	-52%	-	Electrical stimulation
Otsuji, 1993 [2]	EPA ester	1.0	8 weeks	20	-	-	-40% †	NS	Ischemia
Oskarsson, 1993	MaxEPA	1.0	6 weeks	22	-	-	-55%*	NS	90-min ischemia; 30-min reperfusion

VPB = ventricular premature beats/complex; IS = infarct size/size of ischemia zone; AS = arrhythima score (according to Curtis et al, 1987); TSR =length of time in normal sinus rhythm; ARAr = areas at risk of arrhythmias; ISH = ischemia
- Not reported NS = no significant difference compared to controls
* *P*<0.05 compared to controls † p<0.01 compared to controls

[1] See Chapter 2: Methods for the effects expressed as percent change
[2] Study results were biased by excluding more subjects who died from arrhythmias in the control group

Table 3-20. Effects of Intravenously Infused Omega-3 Fatty Acids on Ischemia-Induced or Spontaneous Arrhythmias in Mongrel Dogs

Author, Year	Omega-3 Arms (N)	Dosage	Controls (N)	Results	Experiment Protocols
Billman, 1994	10 ml fish oil conc (n=4), or 5 ml fish oil + 5 ml Tg conc (n=4)	Fish oil conc.: 70% EPA+DHA T conc.: 65% EPA+DHA	Saline (n=3) or lipid emulsion (n=5)	N / VF incidence Fish oil infusion 8 13%* Controls 8 100% *P<0.005 compared to controls	Exercise-ischemia (2-min) test
Billman, 1999	Albumin-bound ALA (n=8) EPA (n=7) DHA (n=8)	98% EPA 91% DHA >99% ALA No data on the amount (ml) infused	SBO lipid emulsion, containing 7%~8% ALA (n=7)	N / VF incidence ALA 8 25%* EPA 7 29%* DHA 8 25%* Controls 7 100% *P<0.05 compared to controls	Exercise-ischemia (2-min) test
Lo, 1991	ALA (n=8) [1]	1, 5, 10, 20, 30, or 60 mg/kg	Control buffer, pH 8.1 (no lipids)	Eight dogs were infused control buffer or different doses of ALA. No events of VT or VPB were observed when infusing control buffer, or ALA up to 10 mg/kg. ALA (mg/kg) 20 30 60 VPC 25% 75%* 88%* VT 13% 38% 63%* *P<0.05 compared to control buffer	Normal condition

Tg = triglyceride; VF = ventricular fibrillation; VPC = ventricular premature complex; VT = ventricular tachycardia; conc = concentrate; SBO = soybean oil

[1] A left atrial injection instead of intravenous injection was used as the route of administration in this study

Table 3-21. Effects of Omega-3 Fatty Acids on Contractile Parameters in Whole Animal Isolated Organ and Cell Studies

Author, Year	Animal Model [Type, Age, Sex]	Exposure Duration (Weeks)	Comparison Groups[a]		Amount of Omega-3 Fatty Acid	Experimental Condition	Agent[b]	Heart Rate	Contractility[c]	IP[d]	Cardiac Work
			Omega-3 Fatty Acid (n)	Control (n)							
RAT											
Chemla, 1995	Myocardium, Adult, Male	4	N-3 (15)	N-3 (16)	15%wt	Ambient	None		NC (FVR)		
Demaison, 1993	Isolated heart, weanling, male	8	LIN (29)	SF (32)	100g/kg	Ambient	None				NC
Heard, 1992	Atrial tissue, adult, male	4	FO+SAF (6-11)	SAF (6-11)	19.5%+0.5%wt	Ambient	ISO		NC (FOC)		
			FO+SAF (6-11)	SAF (6-11)	19.5%+0.5%wt	Ambient	Saline	NC	NC (FOC) NC (df/dt) NC (–df/dt)		
			FO+SAF (6-11)	SAF (6-11)	19.5%+0.5%wt	Ambient	LPS	D*	I* (FOC) I* (df/dt) I* (–df/dt)		
Ku, 1997	Isolated heart, aged female	12	HC+EPA	HC	300mg/kg	Ambient	None	NC			
			HC+DHA	HC	300mg/kg	Ambient	None	NC			
			HC+DHA	HC+EPA	300mg/kg	Ambient	None	NC			
Leifert, 2000	Ventricular myocyte, young adult, male (Gavage)	3	FO (29-36)	LARD (29-36)	35g/d	Ambient	None			NC (DCL) NC (SCL) NC (PCL) NC (PRP)	
			FO (6 animals)	LARD (6 animals)	35g/d	Ambient	ISO		D*		
			FO (6-9 animals)	LARD (6-9 animals)	35g/d	Ambient	FRGS		D*		
Leifert, 2001	Ventricular myocyte, adult male	3	FO (6 animals)	SF (6 animals)	10%wt	Ambient	ISO		D*** D* (Time) NC (#)		
Reig, 1993	Ventricular tissue, young adult, male	5	FO (5)	HF (5)	6%wt	Ambient	None	NC			

Table 3-21. Effects of Omega-3 Fatty Acids on Contractile Parameters in Whole Animal Isolated Organ and Cell Studies

Author, Year	Animal Model [Type, Age, Sex]	Exposure Duration (Weeks)	Comparison Groups[a]		Amount of Omega-3 Fatty Acid	Experimental Condition	Agent[b]	Heart Rate	Contractility[c]	IP[d]	Cardiac Work
			Omega-3 Fatty Acid (n)	Control (n)							
Laustiola, 1986	Atrial myocyte, weanling, male	16	CLO (7-11)	Std (7-11)	10% wt	High O_2	None	D***		D*** (A)	
			CLO (4-11)	Std (4-11)	10% wt	High O_2	NA	NC		NC (A)	
			CLO (4-11)	Std (4-11)	10% wt	Hypoxia	NA	D***		D*** (A)	
			CLO (4-11)	Std (4-11)	10% wt	Reoxygenation	NA	NC		NC (A)	

IP= inotropic parameters; D = decrease; I = increase; NA = not applicable ; NC = no change; ND= no data; * = $p<0.05$ ** = $p<0.01$; *** = $p<0.001$

A = amplitude
CLO = cod liver oil
D = decrease
DCL =diastolic cell length
df/dt =maximum rate of rise of contraction
DHA =decosahexaenoic acid
EPAe =EPA esters
FO = fish oil
FOC =force of contraction
FRGS =free radical generating system
FVR =force-velocity relationship
HC = high cholesterol
HF = high fat
ISO =isoproteronol
LIN = linseed oil
LPS =lipopolysaccharide
PCL =percent cell length
PRP =post rest potentiation
SAF = safflower oil
SF = saturated fat
STD = standard chow

Table 3-22. Effects of Omega-3 Fatty Acids on Basoelectromechanical Parameters in Whole Animal Isolated Organ and Cell Studies

Author, Year	Animal Model [Type, Age, Sex]	Exposure Duration (Weeks)	Comparisons[a]		Amount of Omega-3	Experiment Condition	Agent	VERP	ARP	RRP	QRS	QT	MAP	RDT
			Omega-3 Fatty Acid (n)	Control (n)										
RAT														
Reig, 1993	Ventricular, young adult, male	5	FO+HF (5)	HF (5)	6+31% wt	Ambient	None	D*						
Karmazyn, 1987	Isolated heart weanling male/female	12	CLO (5-9)	STD (5-9)	10% wt	Ischemia Reperfusion	None							NC
RABBIT														
Gillis, 1992	SR vesicles, weanling, ND	6	FO (9)	SAF (9)	10% wt	Ambient	None	NC	NC	NC	NC	NC	NC epi NC endo	

VERP =left ventricular effective refractory period; ARP = absolute refractory period ; RRP =relative refractory period ;
QRS =ventricular conductance time; Qt = electrocardiogram interval ; MAP =monophasic action potential duration ; RDT = developed or resting tension;
D = decrease; I = increase; NA = not applicable ; NC = no change; ND= no data; * = $p<0.05$ ** = $p<0.01$; *** = $p<0.001$

CLO = cod liver oil
D = decrease
endo =endocardial
epi =epicardial
FO = fish oil
HF = high fat
NC =no change
ND =no data
SAF = safflower oil
STD =standard chow
SR =sarcoplasmic reticulum

Table 3-23. Effects of Omega-3 Fatty Acids on Ion Pumps and Ion Movement in Whole Animal Isolated Organ and Cell Studies

Author, Year	Animal Model [Type, Age, Sex]	Feeding Duration (Weeks)	Comparison Groups[a]		Am-mount of Omega-3	Experiment Condition	Agent	Pump[a] Activity	Cys Ca^{2+} Influx	Cys Ca^{2+} Efflux	Cys Ca^{2+} Content	SR Ca^{2+} Content	SR Ca^{2+} Release	SR Ca^{2+} Uptake
			Omega-3 Fatty Acid (N)	Control (N)										
MOUSE														
Croset, 1989b	SR vesicles, weanling, male	2	ALA ester (3)	SAF (3)	0.5%wt	Ambient	None					D*		
			EPA ester (3)	SAF (3)	0.5%wt	Ambient	None	NC SR $_{Ca2+Mg2+}$				D*		
			DHA ester (3)	SAF (3)	0.5%wt	Ambient	None	NC SR $_{Ca2+Mg2+}$				D*		
Swanson, 1989	SR vesicles, weanling, male	2	SAF+FO (3ht)	SAF+CO (3ht)	10% wt	Ambient	None	D* Ca2+Mg2+						D**
Croset, 1989a	SR vesicles, weanling, male	2	DHA ester (10)	STD (10)	0.4 g/100 g	Ambient	None	NC SR $_{Ca2+Mg2+}$						
			DHA ester (10)	STD (10)	0.8 g/100 g	Ambient	None	NC SR $_{Ca2+Mg2+}$						
			DHA ester (10)	STD (10)	4 g/100 g	Ambient	None	NC SR $_{Ca2+Mg2+}$						
	Cardiac, weanling, male	2	DHA ester (10)	STD (10)	0.4 g/100 g	Ambient	Oligomycin	I* $_{Ca2+Mg2+}$						
			DHA ester (10)	STD (10)	0.8 g/100 g	Ambient	Oligomycin	I* $_{Ca2+Mg2+}$						
			DHA ester (10)	STD (10)	4 g/100 g	Ambient	Oligomycin	NC $_{Ca2+Mg2+}$						
RAT														
Benedikts - dottir, 1988	Cardiac, adult male	16	Cod liver (ND)	Corn (ND)	!0% wt	Ambient	None	NC $_{Na+K+}$						
Pepe, 1999	Cardiac, aged & young adults, male	2	Fish oil (5)	Omega-6 (6)	11.7% wt	Ambient	None				NC			
			Fish oil (5)	Omega-6 (6)	11.7% wt	Ambient w/ NorEpi	None				D* total D* aged NC young			
			Fish oil (5)	Omega-6 (6)	11.7% wt	15-minute ischemia; 5-minute reperfusion	None				D* aged D*** young			

Table 3-23. Effects of Omega-3 Fatty Acids on Ion Pumps and Ion Movement in Whole Animal Isolated Organ and Cell Studies

Author, Year	Animal Model [Type, Age, Sex]	Feeding Duration (Weeks)	Comparison Groups[a]		Am-mount of Omega-3	Experiment Condition	Agent	Pump[a] Activity	Cys Ca^{2+} Influx	Cys Ca^{2+} Efflux	Cys Ca^{2+} Content	SR Ca^{2+} Content	SR Ca^{2+} Release	SR Ca^{2+} Uptake
			Omega-3 Fatty Acid (N)	Control (N)										
Taffet, 1993	SR vesicle, young adult, female	3	CO+FO (11-12)	CO (11-12)	17% wt	Ambient	None							D*
			CO+FO (11-12)	CO (11-12)	17% wt	Ambient	Ca2+ 50uM ATP	D* SR Ca2+Mg2+ D* Ca2+						
			CO+FO (11-12)	CO (11-12)	17% wt	Ambient	Ca2+ 50uM ATP+ Ionomycin	D* SR Ca2+Mg2+ D* Ca2+ NC Mg2+						
			CO+FO (11-12)	CO (11-12)	17% wt	Ambient	Ca2+ 1 mM ATP+ Ionomycin	D* Ca2+Mg2+ D* Ca2+ D*Mg2+				D*		
Leifert, 2001	Cardiac, adult, male	3	Fish oil (8)	SFA (8)	10% wt	Ambient					NC	NC		
			Fish oil (8)	SFA (8)	10% wt	Ambient	Caffeine					NC		
			Fish oil (8)	SFA (8)	10% wt	Ambient	DBHQ					NC		I* Ca^{2+} exchanger efflux
			Fish oil (8)	SFA (8)	10% wt	Ambient	ISO				NC			I* Ca^{2+} exchanger or SR efflux
Black, 1989	SR, adult, male (Gavage)	4	FO (6)	STD(6)	0.5 ml/kg/d	Ambient	Ca^{2+}							NC Ca^{2+} transport activity
Karmazyn, 1987	Ventricular, weanling, male/female	12	Cod liver (5-9)	STD (up to 11)	10%wt	20-minute ischemia; 30-minute reperfusion	None		I**	NC				
Maixent, 1999	Cardiac, adult, male	8	Fish oil (4)	STD (4)	0.5 g/kg	Ambient	OUA	NC $Na+K+$						

Table 3-23. Effects of Omega-3 Fatty Acids on Ion Pumps and Ion Movement in Whole Animal Isolated Organ and Cell Studies

Author, Year	Animal Model [Type, Age, Sex]	Feeding Duration (Weeks)	Comparison Groups[a]		Am-mount of Omega-3	Experiment Condition	Agent	Pump[a] Activity	Cys Ca^{2+} Influx	Cys Ca^{2+} Efflux	Cys Ca^{2+} Content	SR Ca^{2+} Content	SR Ca^{2+} Release	SR Ca^{2+} Uptake
			Omega-3 Fatty Acid (N)	Control (N)										
Chen, 1994	Cardiac, adult, male	2	Fish oil (5)	Coconut (5)	10%wt	Ischemia	None				NC			
			Fish oil (5)	Coconut (5)	10%wt	10-minute ischemia; 1-hour reperfution	None				NC			
			Fish oil (5)	Coconut (5)	10%wt	1-hour ischemia; 4-hour reperfusion	None				NC			
Kinoshita, 1994	Cardiac, adult ND	8	EPA ester (6)	STD (ND)	100 mg/kg/d	Ambient	None	$I^{*}_{Ca2+Mg2+ (Vmax)}$ NC_{Km}						
			EPA ester (6)	STD (ND)	100 mg/kg/d	Ischemic	None	$I^{*}_{Ca2+Mg2+ (Vmax)}$ NC_{Km}						
			EPA ester (6)	STD (ND)	100 mg/kg/d	Ambient	OUA	NC_{Na+K+}						
			EPA ester (6)	STD (ND)	100 mg/kg/d	Ischemic	OUA	NC_{Na+K+}						
Honen, 2002	Atrial, adult, male	3	Fish oil (6)	Canola (6)	3 ml/d	Ambient	None		NC					
PIG														
Lamers, 1988	Sarcolemma, weanling, male/female	8	Fish oil (8)	Lard (8)	4.5%w	Ambient	Ca^{2+}	I^{*}_{Ca2+}						
						Ischemia Reper-fusion	Ca^{2+}	I^{*}_{Ca2+}						

Cys= cytosolic; SR= sarcoplasmic reticulum; D = decrease; I = increase; NC = no change; ND= no data; * = $p<0.05$ ** = $p<0.01$; *** = $p<0.001$

ALAe =alpha linoleic acid
ATP =adenosine triphosphate
CO = corn oil
D = decrease
DBHQ =2,4-Di-tert-buytlhydroquinone
DHA =decosahexaenoic acid
EPAe =eicosapentaenoic acid
FO = fish oil
I =increase
ISO =isoproteronol
Mg2+=magnesium
NC =no change
ND =no data
OUA =ouabain
SAF = safflower oil
SFA =saturated fatty acid
STD =standard chow
SR =sarcoplasmic reticulum
uM =micromoles

Table 3-24. Effects of Omega-3 Fatty Acids on Ion Currents In Whole Animal Isolated Organ and Cell Studies

Author, year	Animal Model [Type, Age, Sex]	Expo-sure Duration (weeks)	Comparison Group[a]		Amount of Omega-3	Expt. Con-dition	Agent	I_{Na}	I_{to}	I_{CaL}	I_K	I_{KI}	I_{KUR}
			Omega-3 Fatty Acid (n)	Control (n)									
RAT													
Minarovic, 1998	ventricular myocyte, Young adult, male	2	FO (ND)	HF (ND))	100g/Kg/d	Ambient	None			NC Ac NC InAc NC A			
Leifert, 2000	ventricular myocyte, Young adult, male	3	FO (17-28)	LARD (17-28)	29% Energy	Ambient	None	NC Ac NC InAc	NC Ac NC InAc				

I_{Na}=initial fast current; I_{to}= transient K+ outward current or initial outward current; $I_{Ca.L}$= voltage dependent L-type Calcium current/inward current/calcium sparks; I k= delayed rectifier K+ current; I_{ki}= inward rectifier K+ current; I_{kur}= ultra rapid K+ current; Ac-activation parameter; InAc = inactivation parameter; D = decrease; I = increase; NC = no change; ND= no data; * = $p<0.05$ ** = $p<0.01$; *** = $p<0.001$

A =amplitude
Ac =activation parameter
FO = fish oil
InAc =inactivation parameter
HF = high fat
NC =no change

Table 3-25. Effects of Omega-3 Fatty Acids on Ion Channels In Whole Animal Isolated Organ and Cell Studies

Author, year	Animal Model [Type, Age, Sex]	Exposure Duration (weeks)	Comparison Groups[a]		Amount of omega-3	Experimental Condition	Agent[b]	Binding to the Ca^{2+} Channel
			Omega-3 FA (n)	Con-trol (n)				
RAT								
Gudmunds -dottir, 1991	Ventricular SL, Adult, male	20	CLO (4-5)	CO (4-5)	10% wt	Ambient	NIT	NC K_d NC B_{max}
	Ventricular SL, Aged, male	88	CLO (4-5)	CO (4-5)	10% wt	Ambient	NIT	NC K_d NC B_{max}
	Ventricular SL, Adult & aged, male	20 & 88	CLO (5)	CO (5)	10% wt	Ambient	NIT	D* K_d NC B_{max}
Minarovic, 1997	Ventricular myocytes, Young adult, male	2	FO (ND)	HF (ND)	100g/kg	Ambient	VER	No effect of agent
			FO (ND)	HF (ND)	100g/kg	Ambient	DIL	No effect of agent

D = decrease; I = increase; NC = no change; ND= no data; * = $p<0.05$ ** = $p<0.01$; *** = $p<0.001$

CLO = cod liver oil
CO = corn oil
D = decrease
DIL =diltiazem
FA =fatty acid
FO = fish oil
HF = high fat
NC =no change
ND =no data
NIT =nitrendipine
VER =verapamil

Table 3-26. Effects of Omega-3 Fatty Acids on Arrhythmogenic and Contractile Parameters in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Age, Type]	Exposure Duration	Comparison Groups[a]		Amount of Omega-3	Experimental Condition	Agent[b]	AR[c]	Con-Tractility[d]	IP	tC_{20}	CD_{20}	CD_{80}	$-C_{max}$	$+C_{max}$
			Omega-3 Fatty Acid (n)	Control (n)											
RAT															
Hallaq, 1992	Rat, neonatal, ventricular	1-2 min Free	DHA (6)	STD (6)	5uM	Ambient	None		NC						
			DHA (10)	STD (10)	5uM	Ambient	OUA	P* T*							
			DHA (6)	STD (6)	5uM	Ambient	NIT		B*						
			DHA (4)	STD (4)	5uM	Ambient	BAY		B*						
			DHA (3-4)	STD (3-4)	5uM	Ambient	VER		NB						
			DHA (3-4)	STD (3-4)	5uM	Ambient	DIL		NB						
			EPA (ND)	STD (ND)	5uM	Ambient	OUA	P*							
Jahangiri, 2000	Rat, adult, atrial	7 minFree	EPA (107/7ht)	STD (107/7ht)	10uM	Ambient	ISO		D**						
			DHA (101/5ht)	STD (101/5ht)	10uM	Ambient	ISO		D*						
			DHA m.e. (71/4ht)	STD (71/4ht)	10uM	Ambient	ISO		NC						
Kang & Leaf, 1994	Rat, neonatal, cardiac	3 minFree	ALA (5)	STD (5)	5-10uM	Ambient	None		D*						
			EPA (46)	STD (46)	5-10uM	Ambient	None		D*	NC A					
			EPA (ND)	STD (ND)	5-10uM	Ambient	Var[a]		D*						
			EPA (ND)	STD (ND)	5-10uM	Ambient	Ca^{2+}	P* T*							
			EPA (ND)	STD (ND)	5-10uM	Ambient	OUA	P* T*							
			EPAe.e. (3)	STD (3)	5-10uM	Ambient	None		NC						
			DHA (32)	STD (32)	5-10uM	Ambient	None		D*	NC A					
			DHA (ND)	STD (ND)	5-10uM	Ambient	Ca^{2+}	P* T*							
			DHA (ND)	STD (ND)	5-10uM	Ambient	OUA	P* T*							

Table 3-26. Effects of Omega-3 Fatty Acids on Arrhythmogenic and Contractile Parameters in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Age, Type]	Exposure Duration	Comparison Groups[a]		Amount of Omega-3	Experimental Condition	Agent[b]	AR[c]	Contractility[d]	IP	tC_{20}	CD_{20}	CD_{80}	$-C_{max}$	$+C_{max}$
			Omega-3 Fatty Acid (n)	Control (n)											
Kang & Leaf, 1996	Rat, neonatal, cardiac	3-7 min Free	ALA (5)	STD (5)	10-15uM	Ambient	LPC	P*	D*						
		3-7 min Free	ALA (5)	STD (5)	10-15uM	Ambient	PTC	P* T*							
		3-7 min Free	EPA (5)	STD (5)	10-15uM	Ambient	LPC	P* T*	D*						
		3-7 min Free	EPA (5)	STD (5)	10-15uM	Ambient	PTC	P* T*							
		3-7 min Free	EPA (5)	STD (5)	10-15uM	Ambient	Ca^{2+} Ionophore	P* T*							
		3-7 min Free	EPA (7)	STD (7)	15uM	Ambient	Electrical pacing		D** EA						
		3-7 min Free	DHA (5)	STD (5)	10-15uM	Ambien	LPC	P*	D*						
		3-7 min Free	DHA (5)	STD (5)	10-15uM	Ambient	PTC	P* T*							
Kang, 1995b	Rat, neonatal, cardiac	5 min Free	EPA (4)	STD (4)	8uM	Ambient	Cholera toxin		D^{ND}						
			EPA (5-8)	STD (5-8)	5-10uM	Ambient	ISO	P* T*	D^{ND}						
			EPA (3)	STD (3)	5-10uM	Ambient	ISO+INDO+ BW	P*							
			EPA (5)	STD (5)	5-10uM	Ambient	cAMP	T*							
			DHA (3)	STD (8)	5-10uM	Ambient	ISO+INDO+ BW	P*							
Leifert, 2000	Rat, adult, ventricular	ND Free	DHA (5)	DA (5)	10uM	Ambient	ISO		D**						
			DHA (4)	Stearic A (4)	10uM	Ambient	LPC		D**						
			DHA (4)	Stearic A (4)	10uM	Ambient	ISO		D*						

Table 3-26. Effects of Omega-3 Fatty Acids on Arrhythmogenic and Contractile Parameters in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Age, Type]	Exposure Duration	Comparison Groups[a]		Amount of Omega-3	Experimental Condition	Agent[b]	AR[c]	Contractility[d]	IP	tC_{20}	CD_{20}	CD_{80}	$-C_{max}$	$+C_{max}$
			Omega-3 Fatty Acid (n)	Control (n)											
Li, 1997	Rat, neonatal, cardiac	ND Free	EPA (ND)	STD (ND)	10uM	Ambient	Eico	T*	D*						
MacLeod, 1998	Rat,adult, ventricular	5 min Free	EPA (6-8)	STD (6-8)	1-7.5uM	Ambient	None		I^{ND} TS						
			EPA (6-8)	STD (6-8)	>10uM	Ambient	None		D^{ND} TS						
			DHA (6-8)	STD (6-8)	1-7.5uM	Ambient	None		I^{ND} TS						
			DHA (6-8)	STD (6-8)	>10uM	Ambient	None		D^{ND} TS						
Negretti, 2000	Rat, adult ventricular	ND Free	EPA (6-57)	STD (6-57)	10uM	Ambient	None		D*** F	I*** RCL					
Pepe, 1994	Rat, adult, cardiac	4 min Free	DHA (6)	STD (6)	5 uM	Ambient	None		NC DL NC TA NC VS/DL						
			DHA (6)	STD (6)	5 uM	Ambient	NIT		B* TA B* VS/DL						
			DHA (6)	STD (6)	5 uM	Ambient	ISO		NC TA NC DL						
			DHA (6)	STD (6)	5 uM	Ambient	BAY		B* TA B* VS/DL						
Rodrigo, 1999	Rat, adult, ventricular	10 min Free	EPA (8)	STD (8)	5uM	Ambient	None		D***TS						
	Rat, adult, SSP ventricular	10 min Free	EPA (5)	STD (5)	5uM	Ambient	Ca^{2+}		D* F NC Relax						
			EPA (5)	STD (5)	10uM	Ambient	Ca^{2+}		D* F NC Relax						
Weylandt, 1996	Rat, neonatal, cardiac	3-12min Free	EPA (8)	STD (8)	15uM	Ambient	ISO	T*							
			EPA (12)	STD (12)	15uM	Ambient	Ca^{2+}	D*							
			DHA (8)	STD (8)	15uM	Ambient	ISO	T*							
			DHA (12)	STD (12)	15uM	Ambient	Ca^{2+}	D*							
		3-12 min Free 48 hr Bound	DHA Free (23)	DHA Bound (23)	15uM	Ambient	ISO	T*							
			EPA Free (23)	EPA Bound (23)	15uM	Ambient	ISO	T*							

Table 3-26. Effects of Omega-3 Fatty Acids on Arrhythmogenic and Contractile Parameters in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Age, Type]	Exposure Duration	Comparison Groups[a]		Amount of Omega-3	Experimental Condition	Agent[b]	AR[c]	Con-Tractility[d]	IP	tC_{20}	CD_{20}	CD_{80}	$-C_{max}$	$+C_{max}$
			Omega-3 Fatty Acid (n)	Control (n)											
			DHA Free (10)	DHA Bound (10)	15uM	Ambient	Ca^{2+}	D*							
			EPA Free (10)	EPA Bound (10)	15uM	Ambient	Ca^{2+}	D*							
Courtois, 1992	Rat, neonatal, ventricular	24 hr Bound	SM3-Na-Al (5)	STD (5)	28%ALA+ 30%EPA	Ambient	None		NC				NC	NC	NC
			SM3-Na-Al (5)	STD (5)	28%ALA+ 30%EPA	Ambient	ISO		D*				NC	NC	NC
			SM3-Na-Al (5)	SM6-Na-Al (5)	28%ALA+ 30%EPA	Ambient	None		NC				NC	NC	I**
			SM3-Na-Al (5)	SM6-Na-Al (5)	28%ALA+ 30%EPA	Ambient	ISO		NC				NC	NC	NC
De Jonge, 1996	Rat, neonatal, ventricular	4-5 d Bound	EPA (4)	STD (4)	214uM	Ambient	None		D*						
Durot, 1997	Rat, neonatal, ventricular	4 d Bound	SM3 (6)	SM6 (6)	25uM EPA+ 25 uM DHA-Al	Ambient	None				NC	NC	NC	NC	NC
			SM3 (6)	SM6 (6)	25uM EPA+ 25uM DHA-Al	Hypoxia	None				NC	NC	NC	NC	NC
			SM3 (6)	SM6 (6)	25uM EPA+ 25uM DHA-Al	Reoxy	None				NC	NC	NC	NC	NC
Fournier, 1995	Rat, neonatal, ventricular	4 d Bound	EPA (11)	DHA (11)	100uM	Ambient	None				NC	NC	NC	NC	NC
Grynberg, 1988	Rat, neonatal, ventricular	24 h Bound	SM3 (11)	SM6 (11)	57%ALA +7%LA +0.2% AA-Na-Al	Ambient	None				NC		NC		
			SM3 (11)	SM6 (11)	57%ALA +7%LA +0.2% AA-Na-Al	Hypoxia	None				NC		NC		

Table 3-26. Effects of Omega-3 Fatty Acids on Arrhythmogenic and Contractile Parameters in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Age, Type]	Exposure Duration	Comparison Groups[a]		Amount of Omega-3	Experimental Condition	Agent[b]	AR[c]	Con-Tractility[d]	IP	tC_{20}	CD_{20}	CD_{80}	$-C_{max}$	$+C_{max}$
			Omega-3 Fatty Acid (n)	Control (n)											
			SM3 (11)	SM6 (11)	57%ALA +7%LA +0.2% AA-Na-Al	Reoxy	None				NC		NC		
Grynberg, 1995	Rat, neonatal, ventricular	4 d Bound	EPA-Na-Al (12)	DHA-Na-Al (12)	100uM	Ambient	None		NC F			NC	NC	NC	NC
			EPA-Na-Al (6)	DHA-Na-Al (6)	100uM	Ambient	ISO		D* F				NC		
			EPA-Na-Al (6)	DHA-Na-Al (6)	100uM	Ambient	Phe		NC				NC		
			EPA-Na-Al (6)	DHA-Na-Al (6)	100uM	Ambient	dBcAMP		D*						
Grynberg, 199	Rat, neonatal, ventricular	4 d Bound	EPA-Al (10)	DHA-Al (10)	0.1mM	Ambient	None		NC			NC	NC	NC	NC
		4 d Bound	EPA-Al (10)	DHA-Al (10)	0.1mM	Ambient	Phe		NC						
		4 d Bound	EPA-Al (10)	DHA-Al (10)	0.1mM	Ambient	ISO		D**						
		4 d Bound	EPA-Al (10)	DHA-Al (10)	0.1mM	Ambient	dBcAMP		D**						
Hallaq, 1990	Rat, neonatal	3-5 d Bound	EPA (6)	STD (6)	5uM	Ambient	None		NC	NC A					
			EPA (6)	STD (6)	5uM	Ambient	OUA		D***	I*** A					
Ponsard, 1999	Rat, neonatal, ventricular	4 d Bound	EPA+DHA-Al (13)	STD (13)	5%EPA+ 7%DHA	Ambient	None		NC			NC	NC	NC	NC
			EPA+DHA-Al (7)	N-6 (7)	5%EPA+ 7%DHA	Ambient	ISO		I*			NC	NC	NC	NC
			EPA+DHA-Al (6)	N-6 (6)	5%EPA+ 7%DHA	Ambient	PHE		I*			NC	NC	NC	NC

Table 3-26. Effects of Omega-3 Fatty Acids on Arrhythmogenic and Contractile Parameters in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Age, Type]	Exposure Duration	Comparison Groups[a]		Amount of Omega-3	Experimental Condition	Agent[b]	AR[c]	Contractility[d]	IP	tC_{20}	CD_{20}	CD_{80}	$-C_{max}$	$+C_{max}$
			Omega-3 Fatty Acid (n)	Control (n)											
Reithman, 1996	Rat, neonatal, cardiac	3 d Bound	DHA (15)	STD (15)	60uM	Ambient	NA+TIM	D**							
Weylandt, 1996	Rat, neonatal, cardiac	48 hr Bound	EPA (107)	STD (51)	15uM	Ambient	ISO	NC							
			EPA (20)	STD (14)	15uM	Ambient	Ca^{2+}	NC							
			DHA (51)	STD (13)	15uM	Ambient	ISO	NC							
			DHA (20)	STD (6)	15uM	Ambient	Ca^{2+}	NC							
			EPA (107)	DHA (51)	15uM	Ambient	ISO	NC							
			EPA (6-14)	DHA (6-14)	15uM	Ambient	Ca^{2+}	NC							
GUINEA PIG															
Ferrier, 2002	Guinea pig, adult, ventricular	15-20 min Free	DHA m.e. (18-24)	STD (18-24)	10uM	Ambient	None		D***CICR NC VSRM						
Juan, 1987	Guinea pig, adult, isolated heart	30 min Free	EPA-Na (8)	STD (8)	$6x10^{-8}$ mol/min	Ambient	OvAl	NC							
			EPA-Na (8)	STD (8)	$15x10^{-8}$ mol/min	Ambient	OvAl	D*							
			EPA-Na (5)	STD (5)	$15x10^{-8}$ mol/min	Ambient	OvAl+Es	D*							
MacLeod, 1998	Guinea pig, adult, ventricular	5 min Free	EPA (6-8)	STD (6-8)	5-20uM	Ambient	None		D^{ND} TS dd						
			DHA (6-8)	STD (6-8)	5-20uM	Ambient	None		D^{ND} TS dd						
Rodrigo, 1999	Guinea pig, adult, ventricular	10 min Free	EPA (7)	STD (7)	5uM	Ambient	None		D***TS						
	Guinea pig, adult, SSP ventricular	10 min Free	EPA (5)	STD (5)	5uM	Ambient	Ca^{2+}		D* F NC Relax						

Table 3-26. Effects of Omega-3 Fatty Acids on Arrhythmogenic and Contractile Parameters in Isolated Organ and Cell Culture Studies

AR= arrhthymia; IP= inotropic parameters; tC_{20}= contracting coupling delay; CD_{20}= contraction delay at 20% relaxation ; CD_{80}= contraction delay at 80% relaxation ; $-C_{max}$= relaxation time; $+C_{max}$= cell shortening velocity; D = decrease; I = increase; NC = no change; ND= no data; * = $p<0.05$ ** = $p<0.01$; *** = $p<0.001$

A =amplitude

AI =adequate intake

ALA =alpha linoleic acid

AR =arrhythmia

B =blocked

BAY =Bay K8644

BW = BW 755c lipoxygenase inhibitor

cAMP =cyclic adenosine monophosphate

CICR =calcium induced contractile response

D = decrease

DA =amplitude

dBcAMP =dibutyryl cyclic adenosine monophosphate

DHA m.e. =decosahexaenoic acid methylated

DIL =diltiazem

DL =diastolic length

Eico =eicosanoids

EPA =eicosapentaenoic acid

EPAe.e. = eicosapentaenoic acid ethylated

Es =esculetin

F =frequency

INDO =indomethacin

IP =inotropic parameters

ISO =isoproteronol

LA =linoleic acid

LPC =lysophosphatidylcholine

N-6 =omega-6

NA+TIM =sodium and timolol

NB =no block

NC =no change

ND =no data

NIT =nitrendipine

OUA =ouabain

OvAI =ovalbumin

P =prevented

PHE =phenylephrine

PTC =palmitoylcamitine

RCL =resting cell length

SM3 =synthesized medium for omega-3 group

SM6 = synthesized medium for omega-6 group

STD = standard chow

T =terminated

TA =twitch amplitude

TS =twitch size

uM=micromoles

VER =verapamil

VS/DL=velocity of shortening/diastolic length

VSRM =voltage sensitive release mechanism

Table 3-27. Effects of Omega-3 Fatty Acids on Basoelectromechanical Parameters in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Type, Age]	Exposure Duration	Omega-3 Fatty Acid (n)	Con-trol (n)	Amount of Omega-3	Experi-mental Condition	Agent	AP	APA	APD_{40}	APD_{80}	V_{max}	MDP	OS	Other
RAT															
Bogdanov, 1998	Rat, adult ventricular	10-15 min Free	EPA (ND)	STD (ND)	5-10uM	Ambient	None	I^{ND}	NC						
			EPA (ND)	STD (ND)	20uM	Ambient	None		D^{ND}	I^{ND}		D^{ND}			
			DHA (ND)	STD (ND)	10-50uM	Ambient	None		D^{ND}	I^{ND}		D^{ND}			
Kang, 1995	Rat, neonatal, ventricular	2-5 min Free	EPA (8)	STD (8)	10uM	Ambient	None	D* F D**	NC		D**	NC			
MacLeod, 1998	Rat, adult, ventricular	5 min Free	EPA (11-14)	STD (11-14)	1-7.5uM	Ambient	None				I^{ND} dd				
			EPA (11-14)	STD (11-14)	>10uM	Ambient	None				D^{ND} dd				
			DHA (6-8)	STD (6-8)	1-7.5uM	Ambient	None				I^{ND}				
			DHA (11-14)	STD (11-14)	>10uM	Ambient	None				D^{ND} dd				
Durot, 1997	Rat, neonatal, ventricular	4 d Bound	SM3 (9)	SM6 (9)	25uM EPA+ 25uM DHA-Al	Ambient	None	NC	NC	NC	NC	I*	NC	NC	
			SM3 (5)	SM6 (5)	25uM EPA+ 25uM DHA-Al	Hypoxia	None	NC	D*	D**	D*	NC	NC		
			SM3 (5)	SM6 (5)	25uM EPA+ 25uM DHA-Al	Reoxy	None	NC	NC	NC	NC	NC	Im		
Fournier, 1995	Rat, neonatal, ventricular	4 d Bound	EPA (11)	DHA (11)	100uM	Ambient	None	NC	I*	NC	NC	NC	NC	I*	
Grynberg, 1988	Rat, neonatal, ventricular	24 h Bound	SM3 (11)	SM6 (11)	57%ALA+ 7% LA+ +0.2% AA-Na-Al	Ambient	None	NC	NC	NC	NC	NC	NC	NC	
			SM3 (11)	SM6 (11)	57%ALA+ 7% LA+ +0.2% AA-Na-Al	Hypoxia	None	NC	D**	NC	NC	NC	NC	D*	

Table 3-27. Effects of Omega-3 Fatty Acids on Basoelectromechanical Parameters in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Type, Age]	Exposure Duration	Omega-3 Fatty Acid (n)	Con-trol (n)	Amount of Omega-3	Experi-mental Condition	Agent	AP	APA	APD_{40}	APD_{80}	V_{max}	MDP	OS	Other
			SM3 (11)	SM6 (11)	57%ALA+ 7% LA +0.2% AA-Na-Al	Reoxy	None	NC	I**	NC	NC	NC	NC	I*	
Grynberg, 1996	Rat, neonatal, ventricular	4 d Bound	EPA-Al (10)	DHA-Al (10)	0.1mM	Ambient	None		I*		NC	NC	NC	I*	
Reithman, 1996	Rat, neonatal, cardiac	3 d Bound	DHA (28-29)	STD (28-29)	60uM	Ambient	None	NC	I*						
			DHA (14-19)	STD (14-19)	60uM	Ambient	NA+TIM	D*							
			DHA (10-11)	STD (10-11)	60uM	Ambient	ISO	D*							
			DHA (4)	STD (4)	60uM	Ambient	OUA	D*							
GUINEA PIG															
MacLeod, 1998	Guinea pig, adult, ventricular	5 min Free	EPA (12-16)	STD (12-16)	1-20uM	Ambient	None				D^{ND} dd				
			DHA (12-16)	STD (12-16)	1-20uM	Ambient	None				D^{ND} dd				
CAT															
Bayer, 1979	Cat, adult, **heart** in situ	5 min Free IV	ALA-Na (7)	STD (7)	2mg/kg/ min	Ambient	INDO								NC_{AC} NC_{AVC} NC_{ARP} NC_{AVRP}

NC = no change; AP=action potential rate; APA= action potential amplitude; APD_{40}= action potential duration at 40% depolarization; APD_{80}= action potential duration at 80% depolarization; V_{max}= maximum rate of depolarization; MDP= maximum diastolic potential; OS= overshoot; D = decrease; I = increase; NC = no change; ND= no data; * = $p<0.05$ ** = $p<0.01$; *** = $p<0.001$

Table 3-27. Effects of Omega-3 Fatty Acids on Basoelectromechanical Parameters in Isolated Organ and Cell Culture Studies

AA =arachidonic acid
AC =intra-atrial conduction time
ALA = alpha linoleic acid
ARP =functional refractory period of the atrium
AVC =atrioventricular conductance time
AVRP =functional refractory period of atrioventricular conducting system
D = decrease
dd =dose dependent
DHA =decosahexaenoic acid
F =frequency
I =increased
INDO =indomethacin
ISO= isoproteronol
LA =linoleic acid
MDP= maximum diastolic potential
N-6 =omega 6
ND =no data
OS= overshoot
SM3 = synthesized medium for omega-3 group
SM6 = synthesized medium for omega-6 group
STD = standard chow
SR =sarcoplasmic reticulum
uM =micromoles

Table 3-28. Effects of Omega-3 Fatty Acids on Ion Pumps and Ion Movement in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Type, Age]	Exposure Duration	Comparison Groups		Amount of Omega-3	Experimental Condition	Agent	Pump Activity	Cys. Ca^{2+} influx	Cys. Ca^{2+} efflux	Cys Ca^{2+} Content	SR Ca^{2+} Content	SR Ca^{2+} Uptake	SR Ca^{2+} Release	Exchanger	Other
			Omega-3 Fatty Acid (n)	Control (n)												
RAT																
Kang & Leaf, 1996	Rat, neonatal, cardiac	7min Free	EPA (6)	STD (6)	10-15uM	Ambient	None				NCs_{ys} NC_{dia}					
			EPA (6)	STD (6)	10-15uM	Ambient	LPC				T_{CaFlu}					
Negretti, 2000	Rat, ND ventricular	ND Free	EPA (46)	STD (46)	10uM	Ambient	Ca^{2+}				D*** Bas					
			EPA (4)	STD (4)	5uM	Ambient	Caff					I*				
			DHA (3)	STD (3)	5uM	Ambient	Caff					I*				
O'Neill, 2002	Rat, ND ventricular	ND Free	EPA (6)	STD (6)	10uM	Ambient	Ca^{2+}		D*	NC	D^{ND} Bas					
			EPA (12)	STD (12)	10uM	Ambient	Caff			NC						
Pepe, 1994	Rat, young adult, cardiac	4 min Free	DHA (6)	STD (6)	5uM	Ambient	None				NC					
			DHA (6)	STD (6)	5uM	Ambient	NIT		B*		B*					
			DHA (6)	STD (6)	5uM	Ambient	BAY		B*		B*					
			DHA (6)	STD (6)	5uM	Ambient	ISO				NC					
Rinaldi, 2002	Rat, adult, ventricular	20 min vs 3 d Free	DHA+KCl (9)	DHA+KCl (9)	10uM	Ambient	KCl				*D* * mag of I					
			DHA (9)	DHA (9)	10uM	Ambient	KCl				*D* ** mag of I					
			DHA (9)	DHA (9)	10uM	Anoxia	None				D**					
			DHA (9)	DHA (9)	10uM	Anoxia	KCl				D**					
			DHA (9)	DHA (9)	10uM	Anoxia	ET-1				D**					

Table 3-28. Effects of Omega-3 Fatty Acids on Ion Pumps and Ion Movement in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Type, Age]	Exposure Duration	Comparison Groups		Amount of Omega-3	Experimental Condition	Agent	Pump Activity	Cys. Ca^{2+} influx	Cys. Ca^{2+} efflux	Cys Ca^{2+} Content	SR Ca^{2+} Content	SR Ca^{2+} Uptake	SR Ca^{2+} Release	Exchanger	Other
			Omega-3 Fatty Acid (n)	Control (n)												
		20 min free	DHA+ ET-1 (9)	STD (9)	10uM	Ambient	ET-1				I**					
			DHA (9)	STD (9)	10uM	Ambient	ET-1				*D* ** mag of I					
			DHA (9)	STD (9)	10uM	Ambient	None				NC_{bas}					
			DHA +KCl (9)	STD (9)	10uM	Ambient	KCl				I***					
			DHA (9)	STD (9)	10uM	Ambient	KCl				*D* ** mag of I					
			DHA+ET-1 (9)	STD (9)	10uM	Ambient	ET-1				*D* ** mag of I					
			DHA (9)	STD (9)	10uM	Ambient	ET-1				*D* ** mag of I					
			DHA (9)	STD (9)	10uM	Anoxia	None				*D* ** mag of I					
			DHA (9)	STD (9)	10uM	Anoxia	KCl				D**					
			DHA (9)	STD (9)	10uM	Anoxia	ET-1				D**					
		3 d Free	DHA (9)	STD (9)	10uM	Ambient	None				NC_{bas}					
			DHA+KCl (9)	STD (9)	10uM	Ambient	KCl				I***					
			DHA (9)	STD (9)	10uM	Ambient	KCl				*D* ** mag of I					
			DHA (9)	STD (9)	10uM	Anoxia	None				D**					
			DHA (9)	STD (9)	10uM	Anoxia	KCl				D**					
			DHA (9)	STD (9)	10uM	Anoxia	ET-1				D**					

Table 3-28. Effects of Omega-3 Fatty Acids on Ion Pumps and Ion Movement in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Type, Age]	Exposure Duration	Comparison Groups		Amount of Omega-3	Experimental Condition	Agent	Pump Activity	Cys. Ca^{2+} influx	Cys. Ca^{2+} efflux	Cys Ca^{2+} Content	SR Ca^{2+} Content	SR Ca^{2+} Uptake	SR Ca^{2+} Release	Exchanger	Other
			Omega-3 Fatty Acid (n)	Control (n)												
Rodrigo, 1999	Rat, adult, SSP ventricular	10 min Free	EPA (5)	STD (5)	5uM	Ambient	Ca^{2+}							D*		
			EPA (5)	STD (5)	10uM	Ambient	Ca^{2+}							D*		
Vitelli, 2002	Rat, adult, ventricular	20 min Free	DHA (ND)	STD (ND)	10uM	Ambient	Ca^{2+} free KRB				NC_{bas}					
			DHA (ND)	STD (ND)	10uM	Ambient	$CaCl_2$ KRB				NC_{bas}					
			DHA+ DXR (ND)	STD+ DXR (ND)	10uM	Ambient	DXR+ Ca^{2+} free KRB				D**			I*		
			DHA+ DXR (ND)	STD (ND)	10uM	Ambient	DXR+ Ca^{2+} free KRB				NC					
			DHA+ DXR (ND)	DHA (ND)	10uM	Ambient	DXR+ Ca^{2+} free KRB				NC					
			DHA+ DXR (9)	STD+ DXR (9)	10uM	Ambient	DXR+ CaCl2 KRB				D**			I*		
			DHA+ DXR (9)	STD (9)	10uM	Ambient	DXR+ CaCl2 KRB				NC					
			DHA+ DXR (9)	DHA (9)	10uM	Ambient	DXR+ CaCl2 KRB				NC					
			DHA (9)	STD (9)	10uM	Ambient	Caff+ $CaCl_2$ Free KRB				D**			I*		

Table 3-28. Effects of Omega-3 Fatty Acids on Ion Pumps and Ion Movement in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Type, Age]	Exposure Duration	Comparison Groups		Amount of Omega-3	Experimental Condition	Agent	Pump Activity	Cys. Ca^{2+} influx	Cys. Ca^{2+} efflux	Cys Ca^{2+} Content	SR Ca^{2+} Content	SR Ca^{2+} Uptake	SR Ca^{2+} Release	Exchanger	Other
			Omega-3 Fatty Acid (n)	Control (n)												
			DHA+ DXR (9)	STD (9)	10uM	Ambient	Caff+$CaCl_2$ Free KRB				NC					
			DHA (9)	STD (9)	10uM	Ambient	Caff+ CaCl2 KRB				D**			I*		
			DHA+ DXR (9)	STD (9)	10uM	Ambient	Caff+CaCl2 KRB				NC					
Xiao, 1997	Rat, adult ventricular	ND Free	EPA (ND)	STD (ND)	1.5uM	Ambient	None									D** calcium transients
			EPA (ND)	STD (ND)	15uM	Ambient	None									D** calcium transients
Hallaq, 1990	Rat, neonatal, cardiac	3-5d Bound	EPA (8)	STD (8)	5uM	Ambient	None				NC					
		3-5d Bound	EPA (3)	STD (3)	5uM	Ambient	OUA (1uM)				NC					
		3-5d Bound	EPA (5)	STD (5)	5uM	Ambient	OUA (0.1mM)				D***					
		3-5d Bound	EPA (10)	STD (10)	5uM	Ambient	OUA (0.1mM)	NC NaK								
		3-5d Bound	EPA (11)	STD (11)	5uM	Ambient	BUME	NC NaK								
		3-5d Bound	EPA (8)	STD (8)	5uM	Ambient	OUA+ BUME	NC NaK								
	Rat, neonatal, ventricular	4d Bound	DHA (4-11)	STD (4-11)	5uM	Ambient	OUA		B* I							
		4d Bound	DHA (5-14)	STD (5-14)	5uM	Ambient	NIT		B^{ND} D							
		4d Bound	DHA+NIT (5-14)	DHA (5-14)	5uM	Ambient	NIT		NC							
		4d Bound	DHA (5-14)	STD (5-14)	5uM	Ambient	BAY		B^{ND} I							

Table 3-28. Effects of Omega-3 Fatty Acids on Ion Pumps and Ion Movement in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Type, Age]	Exposure Duration	Comparison Groups		Amount of Omega-3	Experimental Condition	Agent	Pump Activity	Cys. Ca^{2+} influx	Cys. Ca^{2+} efflux	Cys Ca^{2+} Content	SR Ca^{2+} Content	SR Ca^{2+} Uptake	SR Ca^{2+} Release	Exchanger	Other
			Omega-3 Fatty Acid (n)	Control (n)												
		4d Bound	DHA+ BAY (5-14)	DHA (5-14)	5uM	Ambient	BAY		NC							
		4d Bound	DHA (5-14)	STD (5-14)	5uM	Ambient	OUA + NIT		BND							
		4d Bound	DHA +OUA + NIT (5-14)	DHA (5-14)	5uM	Ambient	OUA + NIT		NC							
		4d Bound	DHA+Bay +NIT (5-14)	STD+Bay +NIT	5uM	Ambient	BAY + NIT		BND							
		4d Bound	DHA+Bay +NIT (5-14)	DHA (5-14)	5uM	Ambient	BAY + NIT		NC							
		4d Bound	EPA (5-14)	STD (5-14)	5uM	Ambient	NIT		BND							
		4d Bound	EPA+NIT (5-14)	EPA (5-14)	5uM	Ambient	NIT		NC							
		4d Bound	EPA (5-14)	STD (5-14)	5uM	Ambient	BAY		BND I							
		4d Bound	EPA+BAY (5-14)	EPA (5-14)	5uM	Ambient	BAY		NC							
		4d Bound	EPA (5-14)	STD (5-14)	5uM	Ambient	OUA + NIT		BND							
		4d Bound	EPA +OUA + NIT (5-14)	EPA (5-14)	5uM	Ambient	OUA + NIT		NC							
		4d Bound	EPA+Bay +NIT (5-14)	STD+Bay +NilT	5uM	Ambient	BAY + NIT		BND							
		4d Bound	EPA+Bay +NIT (5-14)	EPA (5-14)	5uM	Ambient	BAY + NIT		NC							

Table 3-28. Effects of Omega-3 Fatty Acids on Ion Pumps and Ion Movement in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Type, Age]	Exposure Duration	Comparison Groups		Amount of Omega-3	Experimental Condition	Agent	Pump Activity	Cys. Ca^{2+} influx	Cys. Ca^{2+} efflux	Cys Ca^{2+} Content	SR Ca^{2+} Content	SR Ca^{2+} Uptake	SR Ca^{2+} Release	Exchanger	Other
			Omega-3 Fatty Acid (n)	Control (n)												
Rodrigo, 1999	Guinea pig, adult, SSP ventricular	10 min Free	EPA (5)	STD (5)	5uM	Ambient	Ca^{2+}							D*		
DOG																
Philipson, 1985	Dog, adult, ventricular SR vesicles	1.5 sec Free	ALA (9)	STD (9)	30uM	Ambient	Ca^{2+}								$I^*_{NaCa\ exchange}$	
		2 min Free	ALA (3)	STD (3)	20uM	Ambient	Pre-loaded Ca^{2+}									$I^*_{SL\ pass\ Ca\ efflux}$
Philipson, 1987	Dog, adult, ventricular SR vesicles	1.5 sec Free	ALA (3)	STD (3)	60uM	Ambient	Ca^{2+}								$I^*_{NaCa\ exchange}$	
		2 min Free	ALA (4)	STD (4)	30uM	Ambient	Pre-loaded Ca^{2+}									$I^*_{SL\ pass\ Ca\ efflux}$
Goel, 2002	Pig, adult ventricular SR vesicles	90+/-30s Free	ALA (3-5)	STD (3-5)	50uM	Ambient	None								$NC_{Na/H\ exchange}$	
			DHA (3-5)	STD (3-4)	50uM	Ambient	Na^+								$D^*_{Na/H\ exchange}$	
			EPA (3-5)	STD (3-5)	10uM	Ambient	None								$NC_{Na/H\ exchange}$	
			EPA (3-5)	STD (3-5)	25uM	Ambient	None								$NC_{Na/H\ exchange}$	
			EPA (3-6)	STD (3-6)	50uM	Ambient	None								$D^*_{Na/H\ exchaange}$	$NC_{pass\ NA\ efflux}$
			EPA (3-5)	STD (3-5)	100uM	Ambient	None								$D^*_{Na/H\ exchaange}$	
			DHA (3-5)	STD (3-5)	10uM	Ambient	None								$NC_{Na/H\ exchange}$	
			DHA (3-5)	STD (3-5)	25uM	Ambient	None								$D^*_{Na/H\ exchange}$	

Table 3-28. Effects of Omega-3 Fatty Acids on Ion Pumps and Ion Movement in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Type, Age]	Exposure Duration	Comparison Groups		Amount of Omega-3	Experimental Condition	Agent	Pump Activity	Cys. Ca^{2+} influx	Cys. Ca^{2+} efflux	Cys Ca^{2+} Content	SR Ca^{2+} Content	SR Ca^{2+} Uptake	SR Ca^{2+} Release	Exchanger	Other
			Omega-3 Fatty Acid (n)	Control (n)												
			DHA (3-5)	STD (3-5)	50uM	Ambient	None								$D^*_{Na/H}$ exchange	NC_{pass} NA efflux
			DHA (3-5)	STD (3-5)	100uM	Ambient	None								$D^*_{Na/H}$ exchange	

Cys= cytopsolic; SR= sarcoplasmic reticulum; D = decrease; I = increase; NC = no change; ND= no data; * = p<0.05 ** = p<0.01; *** = p<0.001

ALA =alpha linoleic acud
B =blocked
Bas =baseline
BAY = Bay K8644
BUME =bumetamide
Caff =caffeine
D = decrease
DHA =decosahexaenoic acid
DXR =doxorubicin
EPA =eicosapentaenoic acid
ET-1 =endothelin-1
I =increased
ISO =isoproteronol
KCI =potassium chloride
KRB =Krebs Ringer bicarbonate
LPC =lysophosphatidylcholine
Na =sodium
Na/K =sodium/potassium
NC =no change
ND =no data
NIT =nitrendipine
OUA =ouabain
SL =sarcolemma
SR =sarcoplasmic reticulum
STD = standard chow
uM =micromoles

Table 3-29. Effects of Omega-3 Fatty Acids on Ion Currents in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Type, Age]	Exposure Duration	Comparison Groups		Amount of Omega-3	Experi-mental Condition	Agent	I_{Na}	I_{to}	I_{CaL}	I_K	I_{KI}	I_{KUR}
			Omega-3 Fatty Acid (n)	Control (n)									
MOUSE													
Honore, 1994	Mouse, neonatal, ventricular	ND Free	DHA (9)	STD (9)	30uM	Ambient	None					NC	D*
RAT													
Bogdanov, 1998	Rat, adult ventricular	3-12 min Free	EPA (4)	STD (4)	5-10uM	Ambient	None						NC
			EPA (4)	STD (4)	20uM	Ambient	None						D*
			EPA (4)	STD (4)	50uM	Ambient	None		D*			NC	D*
			DHA (ND)	STD (ND)	5uM	Ambient	None		D^{ND} D^{ND} A I^{ND} delay D** t				
			DHA (ND)	STD (ND)	5uM	Ambient	INDO		D^{ND} D^{ND} A I^{ND} delay D** t				
			DHA (ND)	STD (ND)	50uM	Ambient	None					NC	D^{ND}
Leifert, 1999	Rat, adult ventricular	4 min Free	ALA (6)	STD (6)	25uM	Ambient	None None	+ve Ac*** -ve InAc**					
			EPA (10)	STD (10)	25uM	Ambient	None	+ve Ac*** -ve InAc**					
			DHA (7)	STD (7)	25uM	Ambient	None	D^{ND} A +ve Ac** -ve InAc**					
Macleod, 1998	Rat, adult ventricular	5 min Free	EPA (6-8)	STD (6-8)	5,10,20uM	Ambient	None	D^{ND} A dd		D^{ND} dd			
			EPA (5-8)	STD (5-8)	0.1-10uM	Ambient	None		D^{ND} dd				
			EPA (ND)	STD (ND)	2uM	Ambient	None				D^{ND}	D^{ND}	
			EPA (ND)	STD (ND)	5uM	Ambient	None				D^{ND}	D^{ND}	
			DHA (6-8)	STD (6-8)	5,10,20uM	Ambient	None	D^{ND} A dd					

Table 3-29. Effects of Omega-3 Fatty Acids on Ion Currents in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Type, Age]	Exposure Duration	Comparison Groups		Amount of Omega-3	Experi-mental Condition	Agent	I_{Na}	I_{to}	I_{CaL}	I_K	I_{KI}	I_{KUR}
			Omega-3 Fatty Acid (n)	Control (n)									
			DHA (5-8)	STD (5-8)	5,7.5,10uM	Ambient	None			D^{ND} dd			
			DHA (5-8)	STD (5-8)	0.1-10uM	Ambient	None		D^{ND} dd				
Negretti, 2000	Rat, adult, ventricular	3 min Free	EPA (5)	STD (5)	10uM	Ambient	None			D* A			
			DHA (5)	STD (5)	10uM	Ambient	None			D* A			
O'Neill, 2002	Rat, ND ventricular	ND Free	EPA (6)	STD (6)	10uM	Ambient	Ca^{2+}		D***F I***A				
Pepe, 1994	Rat, adult, cardiac	4 min Free	DHA (6/gp)	STD (6/gp)	5uM	Ambient	None			NC A			
			DHA (6/gp)	STD (6/gp)	5uM	Ambient	NIT			I* A			
			DHA (6/gp)	STD (6/gp)	5uM	Ambient	BAY			B* A			
			DHA (6/gp)	STD (6/gp)	5uM	Ambient	ISO			NC A			
Rodrigo, 1999	Rat, adult ventricular	10 min Free	EPA (8)	STD (8)	5uM	Ambient	None			D***			
Xiao, 1995	Rat, neonatal, ventricular	ND Free	ALA (5)	STD (5)	10uM	Ambient	None	D*					
			EPA (6-10)	STD (6-10)	5-10uM	Ambient	None	D* NC IVC NC Ac NC InAc					
			EPA (4-10)	STD (4-10)	10-40uM	Ambient	None	D* dd					
			EPA (10)	STD (10)	5-10uM	Ambient	None	D** tdv dependent					
			EPA (21)	STD (21)	10uM	Ambient	None	D***					
			DHA (7)	STD (7)	10uM	Ambient	None	D*					
			DHA (7)	STD (7)	5uM	Ambient	None	D**					
Xiao, 1997	Rat, neonatal, ventricular	ND Free	ALA (5)	STD (5)	5uM	Ambient	None			D** -ve IAC			

Table 3-29. Effects of Omega-3 Fatty Acids on Ion Currents in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Type, Age]	Exposure Duration	Comparison Groups		Amount of Omega-3	Experi-mental Condition	Agent	I_{Na}	I_{to}	I_{CaL}	I_K	I_{KI}	I_{KUR}
			Omega-3 Fatty Acid (n)	Control (n)									
		ND Free	EPA (ND)	STD (ND)	0.1-40uM	Ambient	None			D* dd tdv dependent			
		ND Free	EPA (5)	STD (5)	5uM	Ambient	None			D** -ve IAC			
		ND Free	DHA (6)	STD (6)	5uM	Ambient	None			D** -ve IAC			
		ND Free	EPA (11)	STD (11)	1.5uM	Ambient	None			D* NC IVC -ve Shift IAC			
		ND Free	EPA (5)	STD (5)	1uM	Ambient	None			D**			
		ND Free	EPA (8)	STD (8)	5uM	Ambient	None			D**			
GUINEA PIG													
Ferrier, 2002	Guinea pig, adult, ventricular	20 min Free	DHAm.e (18-24)	STD (18-24)	10uM	Ambient	None			I**			
Macleod, 1998	Guinea pig adult, ventricular	5 min Free	EPA (8-10)	STD (8-10)	5,10,20uM	Ambient	None	D^{ND} A dd					
			EPA (5-8)	STD (5-8)	2, 5uM	Ambient	None				D^{ND}	D^{ND}	
			EPA (5-8)	STD (5-8)	5,7.5,10uM	Ambient	None			D^{ND} A dd			
			DHA (8-10)	STD (8-10)	5,10,20uM	Ambient	None	D^{ND} A dd					
			DHA (6-10)	STD (6-10)	5, 7.5, 10uM	Ambient	None			D^{ND} dd			
Rodrigo, 1999	Guinea pig, adult, ventricular	10 min Free	EPA (11)	STD (11)	5uM	Ambient	None			D***			
FERRET													
Xiao, 2002	Ferret, adult, ventricular	ND Free	ALA (7)	STD (7)	5uM	Ambient	None				D**		
			ALA (4-8)	STD (4-8)	10uM	Ambient	None		D*		D***	NC	
			EPA (6)	STD (6)	5uM	Ambient	None				D*		

Table 3-29. Effects of Omega-3 Fatty Acids on Ion Currents in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Type, Age]	Exposure Duration	Comparison Groups		Amount of Omega-3	Experi-mental Condition	Agent	I_{Na}	I_{to}	I_{CaL}	I_K	I_{Kl}	I_{KUR}
			Omega-3 Fatty Acid (n)	Control (n)									
			EPA (4-8)	STD (4-8)	10uM	Ambient	None		D**		D***	NC	
			DHA (7-12)	STD (7-12)	10uM	Ambient	None				D*		
			DHA (6)	STD (6)	0.2-50uM	Ambient	None				D*dd		
			DHA (6-12)	STD (12)	5uM	Ambient	None				D*	NC	
			DHA (5-8)	STD (5-8)	10uM	Ambient	None		D***		D*	NC	
			DHA (5)	STD (5)	10uM	Ambient	Sta				D*		
			DHA (2-6)	STD (2-6)	20uM	Ambient	None				D**	NC	
			DHA (11)	STD (11)	50uM	Ambient	None				D***		

INA= sodium current; ITO= transient K+ outward current or initial outward current; ICA.L= voltage dependent L-type calcium current/inward calcium current/calcium sparks; IK=delayed rectifier K+ current; IKl= inward recitifer K+ current or tail current; IKUR= ultra rapid K+ current; D = decrease; I = increase; NC = no change; ND= no data;
* = $p<0.05$ ** = $p<0.01$; *** = $p<0.001$

A =amplitude
Ac =activation parameter
ALA =alpha linoleic acid
BAY =Bay K8644
D = decrease
dd =dose dependent
DHA =decosahexaenoic acid
D^{nd} =
EPA =eicosapentaenoic acid
F =frequency
I =increased
IAC =
InAc =inactivation parameter
I^{nd} =
INDO =indomethacin
ISO =isoproteronol
IVC =
NC =no change
ND =no data
NIT =nitrendipine
Sta =standard
STD = standard chow
tdv =
uM =micromoles

Table 3-30. Effects of Omega-3 Fatty Acids on Ion Channels in Isolated Organ and Cell Culture Studies

Author, Year	Model [Animal, Age, Type]	Exposure Duration: Free or Bound	Omega-3 Fatty Acid (n)	Con-trol (n)	Amount of Omega-3	Experi-mental Condition	Agent	Na^+ Channel	Cloned Kv1.5 K+ channels	Nitrendipine Binding To Putative Dihydropyridine Sensitive Ca2+ Channels
MOUSE										
Honore, 1994	Mouse, neonatal, ventricular	ND Free	DHA (5-11)	STD (5-11)	30uM	Ambient	None		B* activity	
			ALA (ND)	STD (ND)	ND	Ambient	None		NC activity	
RAT										
Hallaq, 1992	Rat, neonatal, ventricular	4 d Bound	EPA (5-10)	STD (5-10)	5uM	Ambient	NIT			D* High Affinity K_d D** High Affinity B_{max} D** Low Affinity K_d D* Low Affinity B_{max}
			DHA (5-10)	STD (5-10)	5uM	Ambient	NIT			D** High Affinity K_d D* High Affinity B_{max} D** Low Affinity K_d D* Low Affinity B_{max}
Kang, 1997	Rat, neonatal cardiac	2-3 d Bound	EPA (4)	STD (4)	20uM	Ambient	None	NC in number		
			EPA (4)	STD (4)	20uM	Ambient	MEX	D* in number B* increase in expression		

B= Block; STD=Control; D=decrease; d=days; I= Increase; NC=No change; ND=No data; NIT= Nitrendipine;
MEX= Mexiletine; D = decrease; I = increase; NC = no change; ND= no data; * = $p<0.05$ ** = $p<0.01$; *** = $p<0.001$

B =blocked
Bmax =binding capacity
D = decrease
DHA =decosahexaenoic acid
EPA =eicosapantaenoic acid
Kd =affinity
NC =no change
ND =no data
NIT =nitrendipine
MEX =mexiletine
uM =micromoles
STD =standard

Table 3-31. Comparison of IC50 or EC50 Values in Isolated Organ and Cell Culture Studies

Author, year	Model [Animal, Age, Type]	Exposure Duration	Omega-3 Fatty Acid (n)	Con-trol (n)	Experi-mental Condition	Agent	I_{Na}	I_{to}	I_{CaL}	I_K	TS
Leifert, 1999	Rat, adult, ventricular	4 mins Free	DHA	STD	Ambient	None	6.0 ± 1.2 µM				
			EPA	STD	Ambient	None	16.2 ± 1.3 µM				
			ALA	STD	Ambient	None	26.6 ± 1.3 µM				
Macleod, 1998	Rat, adult, ventricular	5 mins Free	DHA	STD	Ambient	None	12.8 ± 0.8 µM	2.6 ± 0.7 µM	27.9 ± 2.5 µM		63 ± 8.3 µM
			EPA	STD	Ambient	None	7.9 ± 0.6 µM	1.9 ± 0.3 µM	9.4 ± 0.8 µM		51 ± 5.0 µM
	Guinea pig, adult, ventricular	5 mins Free	DHA	STD	Ambient	None	15.7 ± 0.9 µM	34.7 ± 2.6 µM			8.5 ± 1.1 µM
			EPA	STD	Ambient	None	8.9 ± 0.5 µM	8.6 ± 1.5 µM			6.7 ± 2.2 µM
Xiao, 1997	Rat, adult, ventricular	ND Free	EPA	STD	Ambient	None			2.1 µM		
	Rat, neonatal, ventricular	ND Free	EPA	STD	Ambient	None			0.8 µM		
Xiao, 2002	Ferret,adult, ventricular	3min Free	DHA	STD	Ambient	None		7.5 µM		20 µM	

D = decrease; I = increase; NC = no change; ND= no data; * = p<0.05 ** = p<0.01; *** = p<0.001

DHA = decosahexaenoic acid
EPA = eicosapantaenoic acid
ND = no data
STD = standard chow
TS =twitch size
uM =micromoles

Appendix A.

A.1 Primary Search Strategy

1. exp cardiovascular diseases/
2. Adhesion molecule expression.mp.
3. Angiographic progression.mp.
4. Angioplast$.mp.
5. (atherogen$ or antiartherogen$).mp.
6. (arrhythmi$ or Antiarrhythmi$).mp.
7. Antithrombo$.mp.
8. endotheli$.mp.
9. exp endothelium, vascular/
10. Beta-thromboglobulin.mp.
11. Cardi$.mp.
12. CHD.mp.
13. Coronary.mp.
14. Hypotens$.mp.
15. Hypotriglyceridem$.mp.
16. heart disease$.mp.
17. Myocardial infarct$.mp.
18. Platelet adhesi$.mp.
19. (postprandial adj (lipemia or lipoprotein$)).mp.
20. Pulmonary Embol$.mp.
21. Heart failure$.mp.
22. Arteriosclerosi$.mp.
23. cardioprotect$.mp.
24. Homocystine/
25. exp Homocysteine/
26. homocyst$.mp.
27. Cystine/
28. cystine.mp.
29. exp Acute-Phase Proteins/
30. acute phase protein$.mp.
31. Acute-Phase Reaction/
32. acute phase react$.mp.
33. exp Blood Coagulation Factor Inhibitors/
34. exp Blood Coagulation Factors/
35. blood coagulation factors$.mp.
36. exp Cell Adhesion Molecules/
37. cell adhesion molecule$.mp.
38. exp Interleukins/
39. interleukin$.mp.
40. Lipid Peroxidation/
41. lipid peroxidat$.mp.

42. exp Hemostasis/
43. hemosta$.mp.
44. haemosta$.mp.
45. exp Diagnostic Techniques, Cardiovascular/
46. or/1-45
47. exp fatty acids, omega-3/
48. fatty acids, essential/
49. Dietary Fats, Unsaturated/
50. linolenic acids/
51. exp fish oils/
52. (n 3 fatty acid$ or omega 3).tw.
53. docosahexa?noic.tw,hw,rw.
54. eicosapenta?noic.tw,hw,rw.
55. alpha linolenic.tw,hw,rw.
56. (linolenate or cervonic or timnodonic).tw, hw,rw.
57. menhaden oil$.tw,hw,rw.
58. (mediterranean adj diet$).tw.
59. ((flax or flaxseed or flax seed or linseed or rape seed or rapeseed or canola or soy or soybean or walnut or mustard seed) adj2 oil$).tw.
60. (walnut$ or butternut$ or soybean$ or pumpkin seed$).tw.
61. (fish adj2 oil$).tw.
62. (cod liver oil$ or marine oil$ or marine fat$).tw.
63. (salmon or mackerel or herring or tuna or halibut or seal or seaweed or anchov$).tw.
64. (fish consumption or fish intake or (fish adj2 diet$)).tw.
65. diet$ fatty acid$.tw.
66. or/47-65
67. dietary fats/
68. (randomized controlled trial or clinical trial or controlled clinical trial or evaluation studies or multicenter study).pt.
69. random$.tw.
70. exp clinical trials/ or evaluation studies/
71. follow-up studies/ or prospective studies/
72. or/68-71
73. 67 and 72
74. (Ropufa or MaxEPA or Omacor or Efamed or ResQ or Epagis or Almarin or Coromega).tw.
75. (omega 3 or n 3).mp.
76. (polyunsaturated fat$ or pufa or dha or epa or long chain or longchain or lc$).mp.
77. 75 and 76
78. 66 or 73 or 74 or 77
79. 46 and 78
80. limit 79 to (addresses or bibliography or biography or congresses or dictionary or directory or editorial or festschrift or government publications or interview or lectures or legal cases or legislation or

letter or news or newspaper article or patient education handout or periodical index or review of reported cases)
81. 79 not 80
82. limit 81 to human
83. (guidelines or practice guideline or meta analysis or review or revewi, academic or review, tutorial or review literature).pt.
84. 82 and 83
85. limit 84 to english language
86. 84 not 85
87. (random$ or rct$).tw.
88. exp randomized controlled trials/
89. exp random allocation/
90. exp double-blind method/
91. exp single-blind method/
92. randomized controlled trial.pt.
93. clinical trial.pt.
94. (clin$ adj trial$).tw.
95. ((singl$ or doubl$ or trebl$ or tripl$) adj (blind$ or mask$)).tw.
96. exp placebos/
97. placebo$.tw.
98. exp comparative study/
99. exp clinical trials/
100. follow-up studies/
101. (follow up or followup).tw.
102. exp case-control studies/
103. (case adj20 control).tw.
104. exp longitudinal studies/
105. longitudinal.tw.
106. exp cohort studies/
107. cohort.tw.
108. exp prospective studies/
109. exp evaluation studies/
110. or/87-109
111. (82 and 110) not 83
112. limit 111 to english language
113. 111 not 112
114. 82 not (111 or 83)
115. limit 114 to english language
116. 114 not 115

A.2 Diabetes Search Strategy

1. exp fatty acids, omega-3/
2. fatty acids, essential/
3. Dietary Fats, Unsaturated/
4. linolenic acids/
5. exp fish oils/
6. (n 3 fatty acid$ or omega 3).tw.
7. docosahexa?noic.tw,hw,rw.
8. eicosapenta?noic.tw,hw,rw.
9. alpha linolenic.tw,hw,rw.
10. (linolenate or cervonic or timnodonic).tw,hw,rw.
11. (mediterranean adj diet$).tw.
12. ((flax or flaxseed or flax seed or linseed or rape seed or rapeseed or canola or soy or soybean or walnut or mustard seed) adj2 oil$).tw.
13. (walnut$ or butternut$ or soybean$ or pumpkin seed$).tw.
14. (fish adj2 oil$).tw.
15. (cod liver oil$ or marine oil$ or marine fat$).tw.
16. (salmon or mackerel or herring or tuna or halibut or seal or seaweed or anchov$).tw.
17. (fish consumption or fish intake or (fish adj2 diet$)).tw.
18. diet$ fatty acid$.tw.
19. menhaden oil$.tw,hw,rw.
20. or/1-19
21. dietary fats/
22. (randomized controlled trial or clinical trial or controlled clinical trial or evaluation studies or multicenter study).pt.
23. random$.tw.
24. exp clinical trials/ or evaluation studies/
25. follow-up studies/ or prospective studies/
26. or/22-25
27. 21 and 26
28. (Ropufa or MaxEPA or Omacor or Efamed or ResQ or Epagis or Almarin or Coromega).tw.
29. (omega 3 or n 3).mp.
30. (polyunsaturated fat$ or pufa or dha or epa or long chain or longchain or lc$).mp.
31. 29 and 30
32. or/20,27-28,31
33. limit 32 to (addresses or bibliography or biography or congresses or dictionary or directory or editorial or festschrift or government publications or interview or lectures or legal cases or legislation or letter or news or newspaper article or patient education handout or periodical index or review of reported cases)
34. Case Report/
35. 32 not (33 or 34)
36. exp Diabetes Mellitus/
37. diabet$.af.
38. 35 and (36 or 37)
39. limit 38 to human

40. limit 39 to english language
41. limit 40 to (guideline or meta analysis or review or review, academic or review, multicase or review, tutorial or review literature)
42. 40 not 41

A.3 Nut Search Strategy

1. exp Nuts/	964
2. exp Cardiovascular Diseases/	1123117
3. (nut or nuts).tw.	1762
4. 1 or 3	2318
5. 4 and 2	145
6 limit 5 to (human and english language)	122

A.4 Risk Factor Update Search Strategy

1. exp fatty acids, omega-3/
2. exp fish oils/
3. (n 3 fatty acid$ or omega 3).tw.
4. docosahexa?noic.tw,hw,rw.
5. eicosapenta?noic.tw,hw,rw.
6. alpha linolenic.tw,hw,rw.
7. (linolenate or cervonic or timnodonic).tw,hw,rw.
8. (fish adj2 oil$).tw.
9. or/1-8
10. limit 9 to human
11. limit 10 to english language
12. exp "Lipoprotein(a)"/
13. c-reactive protein.mp.
14. insulin.mp.
15. exp Factor VIII/
16. exp von Willebrand Factor/
17. heart rate variab$.mp.
18. ankle brachial index.mp.
19. ankle-arm blood pressure index.mp.
20. exp Hemoglobin A, Glycosylated/
21. glycohemoglobin hgb a1c.mp.
22. hgb a1c.mp.
23. exp Apolipoproteins B/
24. apolipoprotein b-100.tw.
25. intima media thickness.mp.
26. carotid doppler.mp.
27. exp Heart Function Tests/
28. exp PLETHYSMOGRAPHY/
29. exp Ultrasonography, Doppler/
30. glycated hemoglobin.mp.
31. or/12-30
32. 11 and 31

Appendix B
Whole Animal Result Form

Animal Characteristic

Subjects and Controls

(Give brief descriptions for each groups. Control group is the group with No intervention or Placebo; or the group with lowest amount of N-3 intakes)

Initial number of animals used: ☐ ND

Number of groups used: ☐ ND

Control group:

Tx Arm 1:

Tx Arm 2:

Tx Arm 3:

Tx Arm 4:

Comments on Subjects and Controls and/or Study Designs

Animals' Diets

Diet composition of the reference diet:

Total fat: ☐ ND

Saturated fatty acids (SFA): ☐ ND

Monounsaturated fatty acids (MUFA): ☐ ND

Polyunsaturated fatty acids (PUFA): ☐ ND

ALA (18:3n-3): ☐ ND

EPA (20:5n-3): ☐ ND

DPA (22:5n-3): ☐ ND

DHA (22:6n-3): ☐ ND

Only EPA+DHA: ☐ ND

Other n-3 FA reporting: ☐ ND

Comments on Animals' Reference/Baseline Diets:

Control Group Characteristics

Control Group

Control (No intervention or Placebo) -- Number enrolled: ☐ ND

Mean Age: ☐ ND

+/- SD/SE: ☐ ND

Age Range: to ☐ ND

Sex of control animals? ☐ ND

- ☐ Only males
- ☐ Only Females
- ☐ Males and Females (give numbers of % distribution)

Control's mean/median age? ☐ ND

Are ages different between groups? ☐ ND

- ☐ No
- ☐ Yes (give brief description)

Is sex different between groups? ☐ ND

- ☐ No
- ☐ Yes (give brief description)

Are body weight different between groups? ☐ ND

- ☐ No
- ☐ Yes (give brief description)

Is control group's diet same as reference diet? ☐ ND

- ☐ Yes
- ☐ No

If controls' diet is NOT reference diet, what is the composition of controls' diet?

Total fat: ☐ ND

Saturated fatty acids (SFA): ☐ ND

Monounsaturated fatty acids (MUFA): ☐ ND

Polyunsaturated fatty acids (PUFA): ☐ ND

ALA (18:3n-3): ☐ ND

EPA (20:5n-3): ☐ ND

DPA (22:5n-3): ☐ ND

DHA (22:6n-3): ☐ **ND**

Only EPA+DHA: ☐ **ND**

Other n-3 FA reporting: ☐ **ND**

Comments on Control Animals' Diets:

Tx Arm No.

DUPLICATE THIS SECTION FOR EACH TREATMENT ARM

Do Not Use The Template (titled Tx Arm No.) to Enter Data.

Name each new section by an appropriate Brief Description (eg, Fish Oil, O3 Diet)

Number each new section's Section ID Tx Arm number from the ANIMAL CHARACTERISTICS section

Treatment Arm #:

Number of animals in Tx Arm #:

Tx Arm's Diets

Diet composition of the Tx Arm's diet:

Total fat: ☐ ND

Saturated fatty acids (SFA): ☐ ND

Monounsaturated fatty acids (MUFA): ☐ ND

Polyunsaturated fatty acids (PUFA): ☐ ND

ALA (18:3n-3): ☐ ND

EPA (20:5n-3): ☐ ND

DPA (22:5n-3): ☐ ND

DHA (22:6n-3): ☐ ND

Only EPA+DHA: ☐ ND

Other n-3 FA reporting: ☐ ND

Tx Arm's Outcomes

Check ND if no outcome reported for this Tx Arm. DO NOT skip any outcome.

Tx Arm # vs. Controls

Effect observed for Outcome 1: ☐ ND

Number of animals: ☐ ND

% of change: ☐ ND

☐ ND

Effect observed for Outcome 2: ☐ ND

Number of animals: ☐ ND

% of change: ☐ ND

☐ ND

Effect observed for Outcome 3: ☐ ND

Number of animals: ☐ ND

% of change: ND

ND

Effect observed for Outcome 4: ND

Number of animals: ND

% of change: ND

ND

Effect observed for Outcome 5: ND

Number of animals: ND

% of change: ND

ND

Effect observed for Outcome 6: ND

Number of animals: ND

% of change: ND

ND

Other outcomes or comments for outcomes (Tx Arm # vs. Controls):

Other Comparisons of Outcomes

DUPLICATE THIS SECTION FOR EACH COMPARISONS OTHER THAN COMPARING TO CONTROLS

Do Not Use The Template (titled Tx Arm 1 vs. 2) to Enter Data.

Comparison groups:

Check ND if no outcome reported for this Tx Arm. DO NOT skip any outcome.

Effect observed for Outcome 1: ☐ ND

Number of animals: ☐ ND

% of change: ☐ ND

☐ ND

Effect observed for Outcome 2: ☐ ND

Number of animals: ☐ ND

% of change: ☐ ND

☐ ND

Effect observed for Outcome 3: ☐ ND

Number of animals: ☐ ND

% of change: ☐ ND

☐ ND

Effect observed for Outcome 4: ☐ ND

Number of animals: ☐ ND

% of change: ☐ ND

☐ ND

Effect observed for Outcome 5: ☐ ND

Number of animals: ☐ ND

% of change: ☐ ND

☐ ND

Effect observed for Outcome 6: ☐ ND

Number of animals: ☐ ND

% of change: ☐ ND

☐ ND

Other outcomes or comments for outcomes:

Appendix C

Evidence Table 1: Whole Animal Studies

Part 1

Author, yr	Study Characteristics	Animal Model	Exposure Duration	Ref. Diet	Dietary Characteristics							
					Groups	Total Fat	% of Total Fatty Acids					Other
							ALA	E+D	n-6	SFA	MUFA	
Abeywardena, 1995	Country: Australia Animal: Wistar rats Funding: Industry	Mean age: ND Age grp: ND Sex: Males	9 months	Standard rat chow (Milling Industries, Adelaide, Australia)	SSO SBO FO (MaxEPA)	15* %w/w or 32* %kcal	0.9 2.8 1.4	0 0 22	59* 44* 7*	13* 21* 28*	22* 25* 15*	
al Makdessi, 1995	Country: Germany Animal: Wistar rats Funding: ND	Mean age: ND Age grp: Young Sex: Males	10 weeks	Low-fat (<1% w/w) standard chow from (Altromin GmbH & Co.)	FO (sardine oil) Coconut oil	10% w/w	1. 3 0.9	29 0	ND ND	31* >60*	ND ND	
Anderson, 1996	Country: Australia Animal: Sprague-Dawley rats Funding: Government	Mean age: ND Age grp: Adult Sex: Males	8 weeks	Total fat: 3.5%	FO (MaxEPA) Safflower oil	Initially given at 0.6 ml and + 0.1 ml/wk up to 1.0 ml w/ increasing body weight	ND 0	ND 0	0 75	10 25	28 15	Total n3 = 41%
Billman, 1994	Country: US Animal: Mongrel dogs Funding: Government	Mean age: ND Age grp: ND Sex: ND	Infusion study	ND	Saline (n=3) or I.V. infusion (n=5) Emulsion of fish oil	100 ml of Intralipid, a 10% lipid emulsion 10 ml FO concentrate (n=4) 5 ml same FO concentrate + 5 ml TG concentrate (n=4)	7 ND ND	0 70 65	ND ND ND	ND ND ND	ND ND ND	

Appendix C

Evidence Table 1: Whole Animal Studies

Part 1

Author, yr	Study Characteristics	Animal Model	Exposure Duration	Ref. Diet	Dietary Characteristics							
					Groups	Total Fat	% of Total Fatty Acids					Other
							ALA	E+D	n-6	SFA	MUFA	
Billman, 1999	Country: US Animal: Mongrel dogs Funding: Government	Mean age: ND Age grp: ND Sex: ND	Infusion study	ND	SBO lipid emulsion (n=7) or saline (n=7)	ND	7~8 in SBO	0	ND	ND	ND	
					EPA		0	E=98 D=1	ND	ND	ND	
					DHA		0	E=1 D=91	ND	ND	ND	
					ALA		>99	0				
Charnock, 1992	Country: Australia Animal: Wistar rats Funding: ND	Mean age: > 3 years old Age grp: Adult Sex: ND	30 months	ND	SSO	12* %w/w or 28 %kcal	1.1	0	ND	23*	23*	N3/n6 = 0.02
					FO		1.4	20	ND	29*	26*	= 2.0
Charnock, 1991	Country: Australia Animal: Wistar rats Funding: ND	Mean age: near 1 yr old Age grp: Adult Sex: males	12 months	NDMilling Industries Australia. Total fat: 3 %w/w	SF/SSO	16* %w/w or 35 %kcal	ND	ND	19	45	29	TT n-3 =1.1 TT n6 =19
					SF/FO		ND	ND	12	41	29	TT n-3 =13 TT n6 =12
Chen, 1994	Country: Taiwan Animal: rabbits Funding: Government	Mean age: ND Age grp: ND Sex: Males	2 weeks.	Standard rabbit chow (Purina 5321, St. Louis, MO, USA)	HC (1% CHOL-enriched diet)	40 %kcal (1% chol)	ND	ND	ND	ND	ND	
					HCF (1% CHOL and 10% FO)	40% energy (1% chol +10% fish oil)	ND	52	ND	ND	ND	

Appendix C

Evidence Table 1: Whole Animal Studies

Part 1

Author, yr	Study Characteristics	Animal Model	Exposure Duration	Ref. Diet	Dietary Characteristics							
					Groups	Total Fat	% of Total Fatty Acids					Other
							ALA	E+D	n-6	SFA	MUFA	
Culp, 1980	Country: US Animal: Mongrel dogs Funding: Government	Mean age: ND Age grp: ND Sex: ND	36 to 45 days	Standard dog chow (Friskies Dinner)	Standard dog chow FO (Menhaden)	ND +25 %kcal	ND ND	0. 1 13	ND ND	32 32	34 20	
Germain, 2003	Country: France Animal: Sprague-Dawley rats Funding: Government	Mean age: ND Age grp: ND Sex: Females	>= 3 weeks	APAE, Jouy en Josas, France.	Palm oil DHASCO (DHA fom purified TGs)	+15% of total fat	ND ND	ND ND	ND ND	high MUFA level ND	ND ND	
Hartog JM 1987	Country: Netherlands Animal: Yorkshire piglets Funding: Dutch Heart Foundation	Mean age: 5 weeks Age grp: Unclear Sex: ND	16 weeks	ND	Lard fat (9% w/w) ML (4.5% mackerel oil + 4.5% lard fat)	ND	1 1	0 13	ND ND	36 32	ND ND	
Hock, 1990	Country: US Animal: Sprague-Dawley rats Funding: Government	Mean age: ND Age grp: Weanling Sex: ND	4 weeks	Fat-free purified diet	CO (corn oil) MO (Menhaden oil)	12 %kcal or 5 %w/w	1 2	0 20	59 5	14* 33*	25* 27*	n3/n6= 0.02 =6.06
Hock, 1987	Country: US Animal: Sprague-Dawley rats Funding: Government	Mean age: ND Age grp: Adult Sex: Males	4 weeks	Fat-free purified diet	CO (corn oil) MO (Menhaden oil)	12 %kcal or 5 %w/w	1 2	0 21	59 4	14* 31*	25* 27*	n3/n6= 0.02 =7.99

Appendix C

Evidence Table 1: Whole Animal Studies

Part 1

Author, yr	Study Characteristics	Animal Model	Exposure Duration	Ref. Diet	Dietary Characteristics							
					Groups	Total Fat	% of Total Fatty Acids					Other
							ALA	E+D	n-6	SFA	MUFA	
Isensee H 1994	Country: Germany Animal: Wistar rats Design: A Funding: Alfred Teufel-Stiftung research foundation	Mean age: 2 months Age grp: Young Sex: Males	10 weeks	Low-fat (<1 %w/w) basic diet (Altromin GmbH, Lage, Germany)	CO LO (Linseed oil) FO	10 %w/w	1 52 0.3	0 0 29	50 20 12	16 9 31	31 16 26	
Kinoshita, 1994	Country: Japan Animal: Mongrel dogs Diseased: Funding: ND	Mean age: ND Age grp: Adult Sex: ND	8 weeks	Standard diet (Oriental Yeast Co.)	Standard diet EPA ester	ND Mochida Pharmaceutical Co	ND ND	ND 100 mg/kg BW/d	ND ND	ND ND	ND ND	
Lo, 1991	Country: Taiwan Animal: Mongrel dogs Funding: ND	Mean age: ND Age grp: ND Sex: MixSex : "either sex"	Infusion study	Same dogs were infused control buffer or different dosages of ALA.	Control buffer ALA infusion	ND	ND 1, 5, 10, 20, 30, or 60 mg/kg	ND ND	ND ND	ND ND	ND ND	
McLennan, 1996	Country: Australia, Switzerland Animal: spontaneously hypertensive Wistar rats Funding: ND	Mean age: ND Age grp: ND Sex: Males	5 weeks	ND	Olive oil EPA DHA EPA+DHA	5% w/w from olive oil 0.5% from n-3; 4.5% from olive oil	ND ND ND ND	ND E:0.5 w/w D:0.5 w/w ND	ND ND ND ND	ND ND ND ND	ND ND ND ND	

Appendix C

Evidence Table 1: Whole Animal Studies

Part 1

Author, yr	Study Characteristics	Animal Model	Exposure Duration	Ref. Diet	Dietary Characteristics							
					Groups	Total Fat	% of Total Fatty Acids					Other
							ALA	E+D	n-6	SFA	MUFA	
McLennan, 1995	Country: Australia Animal: Sprague-Dawley rats Funding: ND	Mean age: 12 weeks Age grp: Adult Sex: Males	12 weeks	Nonpurified lab rat diet. Total fat = 4% w/w	CAN SBO SSO	15 %w/w or 32 %kcal	8 7 5	0 0 0	21 52 64	12 19 12	60 22 23	N3/n6 = 0.37 = 0.14 =0.008
McLennan, Bridle, 1993	Country: Australia Animal: Marmoset monkeys Funding: Government	Mean age: ND Age grp: Old Sex: 50% Males	16 weeks	Low-fat marmoset diet (Milling Industries, Adelaide, Australia) Total fat = 6% w/w	SF/SSO (8% sheep perirenal fat + 2% SSO) SF/FO (7% SF + 3% FO)	16 %w/w	0.8 0.8	1 11	20 10	48 47	ND ND	N3/n6 = 0.12 =1.25
McLennan, 1993	Country: Australia Animal: Sprague-Dawley rats Funding: International Olive Oil Council	Mean age: 30 weeks Age grp: Old Sex: ND	12 weeks	Basic laboratory diet (Milling Industries, Adelaide, Australia) Total fat = 4% w/w	SSO FO	15 %w/w or 32 %kcal	ND ND	ND ND	56 8	15 40	25 25	Total n3 = 4% =17%
McLennan, 1992	Country: Australia Animal: Marmoset monkeys Funding: ND	Mean age: 2 years Age grp: Unclear Sex: breeding pairs	30 months	Total fat: 4 %w/w SFA: 37.3% MUFA: ND PUFA: 18.3%	SSO TFO (tuna fish oil)	12 %w/w or 29 %kcal	ND ND	ND ND	54 11	23 29	ND ND	ND Total n3 = 23%
McLennan, 1990	Country: Australia Animal: Sprague-Dawley rats Funding: Government	Mean age: 2 months Age grp: Adult Sex: Males	18 months	Standard lab rat diet. Total fat = 4% w/w	SF+SSO SF+TFO	16 %w/w or 35 %kcal	0 ND	0 23	58 9	16 31	ND ND	

Appendix C

Evidence Table 1: Whole Animal Studies

Part 1

Author, yr	Study Characteristics	Animal Model	Exposure Duration	Ref. Diet	Dietary Characteristics							
					Groups	Total Fat	% of Total Fatty Acids					Other
							ALA	E+D	n-6	SFA	MUFA	
McLennan, 1988	Country: Australia Animal: Wistar rats Funding: Government	Mean age: "age-matched" Age grp: Unclear Sex: Males	12 months	Standard lab rat diet. Total fat = 4% w/w	SSO TFO	16 %w/w or 35 %kcal	ND ND	0 23	58 9	ND ND	ND ND	
Oskarsson, 1993	Country: US Animal: Mongrel dogs Funding: ND	Mean age: ND Age grp: ND Sex: “Mixed Sex”	6 weeks	ND	No fish oil Rx MaxEPA	ND	ND ND	ND 0.1 g/kg/d	ND ND	ND ND	ND ND	
Otsuji, 1993	Country: Japan Animal: Mongrel dogs Funding: ND	Mean age: ND Age grp: Adult Sex: MixSex : No data on the distribution	8 weeks	Standard diet prepared by Oriental Yeast Co.	Standard dog chow EPA ester	30 g/kg BW /day 100 mg/kg BW/day	ND ND	ND ND	ND ND	ND ND	ND ND	
Pepe, 1996	Country: Australia Animal: Wistar rats Funding: ND	Mean age: 16 weeks Age grp: Young Sex: Males	16 weeks	Nonpurified diet fed to all rats (Milling Industries, Adelaide, Australia). Total fat = 7.6%	SAT (sheep perirenal fat) FO	15.3% w/w	1. 5 1.2	1 36	8 8	55 25	ND ND	

Appendix C

Evidence Table 1: Whole Animal Studies

Part 1

Author, yr	Study Characteristics	Animal Model	Exposure Duration	Ref. Diet	Dietary Characteristics							
					Groups	Total Fat	% of Total Fatty Acids					Other
							ALA	E+D	n-6	SFA	MUFA	
Yang, 1993	Country: US Animal: Sprague-Dawley rats Funding: Government	Mean age: ND Age grp: ND Sex: Males	5 days	Standard rat nonpurified diet (Purina Mills, St. Louis, MO) Total fat = 5 %kcal	Butter FO (fish oil rich pellets)	17 %kcal	ND ND	ND 32	ND 23	ND 25	ND 15	

* estimated values, not reported in original paper

Types of study design:

A = N-3 PUFAs vs. n-6 PUFAs
B = N-3 PUFAs vs. MUFAs
C = N-3 PUFAs vs. SFAs
D = N-3 PUFAs vs. Standard chows

Appendix C

Evidence Table 1: Whole Animal Studies

Part 2

Author, yr	Outcomes	Experimental Protocols	Results	Comment/ Biases/ Limitations
Abeywardena, 1995	VPB VT (%) VF (%) AS Deaths	Myocardial ischemia and reperfusion model. 5-min regional myocardial ischemia. Arrhythmias induced by reperfusion were assessed during a 10-min period after releasing the occlusion and restoring blood flow.	VPB/10 min [SBO vs. SSO] n=18 vs. 18, p>0.05 Increased +176% Estimated [FO vs. SSO] n= 18 vs. 18, p>0.05 Decrease -13% Estimated [FO vs. SBO] n= 18 vs. 18, Decrease -68% Estimated VT (%) during reperfusion [SBO vs. SSO] n=18 vs. 18, Increased 76%/39% Reported [FO vs. SSO] n=18 vs. 18, Decrease 22%/39% Reported VF (%) during reperfusion [SBO vs. SSO]n= 18 vs. 18, Increased 23%/11% Reported [FO vs. SSO] n= 18 vs. 18, Decrease 5%/11% Estimated AS (severity of arrhythmia) during reperfusion [SBO vs. SSO] n= 18 vs. 18, p<0.05 Increase (sig.), +107% Estimated [FO vs. SSO] n= 18 vs. 18, p>0.05 Decrease, -40% Estimated [FO vs. SBO] n= 18 vs. 18, Decrease -71% Estimated Total Deaths [SBO vs. SSO] n= 18 vs. 18, No change 2/18 vs. 1/18 Reported [FO vs. SSO] n= 18 vs. 18, Decrease 0/18 vs. 1/18 Reported VT (%) during ischemia [SBO vs. SSO] n=: 18 vs. 18, No change 44%/39% Reported [FO vs. SSO] n=: 18 vs. 18, Decrease 6%/39% Reported	Sex diff? No Age diff? ND BW diff? No No statistics were performed for FO vs. SBO comparison in the original study
al Makdessi, 1995	AR (%) SIZ (%)	Arrhythima (AR) was defined as salves of extrasystoles and/or ventricular flutter and fibrillation. The preconditioning = in situ by means of 2 cycles of 3 min left anterior descending coronary artery occlusion - 10 min reperfusion.	[FO vs. HCO] (results in figure) AR (%): n= 8 vs. 5, Decrease AR (%) w/ precondition: n= 8 vs. 6, No change SIZ (%): n= 8 vs. 5, No change SIZ (%) w/ precondition: n= 8 vs. 6, No change	Sex diff? No Age diff? No BW diff? ND No statistics were done for FO vs. HCO comparisons in the original study.

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Evidence Table 1: Whole Animal Studies

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Author, yr	Outcomes	Experimental Protocols	Results	Comment/ Biases/ Limitations
Anderson, 1996	VPB VT (%) VF (%) AS	Myocardial ischemia and reperfusion model. 20-min regional myocardial ischemia. Arrhythmias induced by reperfusion were assessed during reperfusion.	Hearts that experienced instability (VPB, VT, and/or VF) during the equilibration period or sustained VT and/or VF during the last 30 s of the occlusion period were excluded from analysis of reperfusion-induced arrhythmias. [FO vs. SO in reperfusion] VPB: n= 8 vs. 6, p>0.05 No change –31% Estimated VT (%): n= 8 vs. 6, p>0.05, 38%/50% Reported VF (%): n= 8 vs. 6, p>0.05, 13%/33% Reported AS: n= 8 vs. 6, p>0.05 Decrease, -54% Estimated	Sex diff? No Age diff? ND BW diff? ND Sustained VT and/or VF were excluded from the analyses.
Billman, 1994	VF (%)	Exercise-plus-ischemia (2-min occlusion) test. VF was induced in one additional animal by the combination of cocain (1.0 mg/kg i.v.) and the exercise-plus-ischemia test.	Ctrl: saline or I.V. infusion VF (%) Ventricular flutter (which degenerates to VF) was reproducibly induced with each presentation of the control (both saline and Intralipid infusions) exercise-plus-ischemia tests. The cocaine exercise-plus-ischemia test induced a similar response. Data for all animals that developed VF have therefore been combined. [FO infusion vs. Ctrl] n= 8 vs. 8, p<0.005 Decrease (sig.) 12.5%/100% (4 ctrl animals developed VF shortly after the treadmill stopped, whereas 4 animals developed malignant arrhythmias while running) Reported.	Sex diff? ND Age diff? ND BW diff? ND
Billman, 1999	VF (%)	Exercise-plus-ischemia (2-min occlusion) test.	Ctrl: Soybean oil lipid emulsion (containing 7%~8% ALA) VF (%). [EPA infusion vs. Ctrl] n= 7 vs. 7, p=0.0105 Decrease (sig.), 2/7 vs. 7/7 Reported [DHA infusion vs. Ctrl] n= 8 vs. 7, p=0.0035 Decrease (sig.), 2/8 vs. 7/7 Reported [ALA infusion vs. Ctrl] n= 8 vs. 7, p=0.0035 Decrease (sig.), 2/8 vs. 7/7 Reported	Sex diff? ND Age diff? ND BW diff? ND
Charnock, 1992	VFT Sustaned VF (%)	Arrhythmias were induced by electrical stimulation protocol in normoxic and ischemic hearts.	VFT [FO vs. SSO in normoxic)] n=ND, No change [FO vs. SSO in ischemia)] n=ND, No change Sustained VF (%) [FO vs. SSO in normoxic)] n=ND, Decrease 8%/13% Reported [FO vs. SSO in ischemia)] n=ND, Decrease 0%/13% Reported	Sex diff? ND Age diff? ND BW diff? ND The procedures for induced-arrhythmias were not reported in the study, but presumably same as McLennan, 1992 & McLennan, Bridle, 1993.

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Evidence Table 1: Whole Animal Studies

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Author, yr	Outcomes	Experimental Protocols	Results	Comment/ Biases/ Limitations
Charnock, 1991	VT (%) VF (%) VPB/15 min AS	15-min ischemia model	[SF/FO vs. SSO] VT (%) in ischemia: n=10 vs. 10, p>0.05 No Change 70%/100% Reported VF (%) in ischemia: n=10 vs. 10, p<0.05 Decreased (sig.) 0%/60% Reported VPB/15 min in ischemia: n=10 vs. 10, p<0.05 Decreased (sig.) –72% Estimated AS in ischemia: n=10 vs. 10, p<0.05 Decreased (sig.) –59% Estimated	Sex diff? No Age diff? No BW diff? No
Chen, 1994	VPB Deaths	In the short-term ischemia study, the ligation was maintained for 10 min in each rabbit and was followed by a reperfusion for 1 hr. In the long-term ischemia study, the ligation was maintained for 1 hr in each rabbit and was followed by a reperfusion for 4 hr.	VT/VF Deaths (%) during ischemia [HCF vs. HC (short term study)] n= 12 vs. 14, p>0.05 Decrease 8%/21% Reported [HCF vs. HC (long term study)] n= 14 vs. 15, p>0.05 No change 21%/27% Reported VPB (%) during ischemia [HCF vs. HC (short term study)] n= 11 vs. 11, p>0.05 Decrease 18%/36% Reported [HCF vs. HC (long term study)] n= 11 vs. 11, p>0.05 No change 36%/55% Reported VT/VF Deaths (%) during reperfusion [HCF vs. HC (short term study)] n= 11 vs. 11, p>0.05 No change 0%/0% Reported [HCF vs. HC (long term study)] n= 11 vs. 11, p>0.05 No change 0%/0% Reported VPB (%) during reperfusion [HCF vs. HC (short term study)] n= 11 vs. 11, p>0.05 No change 18%/18% Reported [HCF vs. HC (long term study)] n= 11 vs. 11, p>0.05 No change 27%/36% Reported	Sex diff? No Age diff? ND BW diff? ND
Culp, 1980	Sudden death Infarct size (%) VPB	Coronary artery thrombosis induced by electrical stimulations.	Ctrl: standard dog chow [FO vs. Ctrl] n= 10 vs. 17 Sudden death (%): No change 30%/29% Reported Infarct size (%): p=0.08 Decrease -52% Estimated Frequency of ectopic beats rose from < 10% at the beginning of the experiment to about 80% after 19 to 247 hrs of stimulation among controls. In contrast, the fish-oil-fed dogs maintained a more normal ECG pattern, showing less than 30% ectopic beats after 19 hours.	Sex diff? ND Age diff? ND BW diff? No

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Evidence Table 1: Whole Animal Studies

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Author, yr	Outcomes	Experimental Protocols	Results	Comment/ Biases/ Limitations
Hartog JM 1987	VPB VT (%) VF (%) Death	Myocardial ischemia and reperfusion models: 5-min ischemia; 10-min reperfusion.	Ctrl: Lard fat (9% w/w) [ML vs. Ctrl] VPB during occlusion: n= 7 vs. 6, p>0.05 No change, +53% Estimated VPB during reperfusion: n= 7 vs. 6, p<0.05 Decrease (sig.) -65% Estimated VT (%) n= 7 vs. 6, No change 29%/17% Reported (all events were occurred during ischemia) VF (%) n= 7 vs. 6, Increased 3/7 vs. 0/6 Reported Of the three animals in ML group had VF, defibrillation was unsuccessful in one animal, which died of ventricular systole during the fifth reperfusion.	Sexdiff? ND Age diff? No BW diff? ND
Hock, 1990	VF (%) AS Deaths	Myocardial ischemia and reperfusion models: 15-min ischemia; 6-hr and 24-hr reperfusion.	[MO vs. CO] VF (%) in 6-hr model: n= 7 vs. 10, p<0.02 Decrease (sig.) 14%/91% Reported AS in 6-hr model: n= 7 vs. 10 p<0.01 Decrease (sig.) -77% Estimated Total Deaths (%) in 24-hr model: n= 21 vs. 22, p<0.05 Decrease (sig.) 24%/69% Reported	Sex diff? ND Age diff? ND BW diff? No
Hock, 1987	VPB Deaths	Ischemia model. "Acute" left main coronary artery ligation was performed. Arrhythima outcomes were observed 15 min after the acute ligation.	[MO vs. CO] Arrhythmia death (%): n= 13 vs. 14, No change 2/13 vs. 2/14 Reported VPB: n= 11 vs. 12, p>0.05 No change	Sex diff? No Age diff? ND BW diff? No

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Evidence Table 1: Whole Animal Studies

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Author, yr	Outcomes	Experimental Protocols	Results	Comment/ Biases/ Limitations
Isensee H 1994	Time in normal sinus rhythm VF (%) VT (%) SIZ (%)	Myocardial ischemia and reperfusion models: 20-min ischemia; 20-min reperfusion	VT (%) during ischemia [FO vs. CO] n= 9~10/grp, Decrease (sig.) 0% vs. 44% Reported [LO vs. CO] n= 9~10/grp, No change 60%/40% Reported [FO vs. LO] n= 9~10/grp, p<0.05 Decrease (sig.) 0%/60% Reported VF (%) during ischemia (results in figure) [FO vs. CO] n= 9~10/grp, p<0.05 Decrease (sig.) 10% vs. (about 45%) Reported [FO vs. CO w/ Aspirin during ischemia] n= 9~10/grp, p>0.05 No change [LO vs. CO] n= 9~10/grp, p>0.05 No change [LO vs. CO w/ Aspirin during ischemia] n= 9~10/grp, p>0.05 No change [FO vs. LO] n= 9~10/grp, p<0.05 Decrease (sig.) 10% vs. (about 40%) Reported [FO vs. LO w/ Aspirin during ischemia] n= ND, p>0.05 No change VF (%) during reperfusion [FO vs. CO] n= 9~10/grp, p>0.05 No change 40%/67% Reported [LO vs. CO] n= 9~10/grp, No change 60%/67% Reported [FO vs. LO] n= 9~10/grp, No change 40%/60% Reported SIZ (%) at end of ischemia (results in figure) [FO vs. CO] n= 9~10/grp, No change [FO vs. CO w/ Aspirin during ischemia] n= 9~10/grp, p>0.05 No change [LO vs. CO] n= 9~10/grp, p>0.05 No change [LO vs. CO w/ Aspirin during ischemia] n= 9~10/grp, p>0.05 No change [FO vs. LO] n= 9~10/grp, p>0.05 No change [FO vs. LO w/ Aspirin during ischemia] n= 9~10/grp, p>0.05 No change Length of time in normal sinus rhythm after occlusion (results in figure) [FO vs. CO] n= 9~10/grp, p<0.05 Increase (sig.) [FO vs. CO w/ Aspirin during ischemia] n= 9~10/grp, p>0.05 No change [LO vs. CO] n= 9~10/grp, p>0.05 No change [LO vs. CO w/ Aspirin during ischemia] n= 9~10/grp, p>0.05 No change [FO vs. LO] n= 9~10/grp, p<0.05 Increase (sig.) [FO vs. LO w/ Aspirin during ischemia] n= 9~10/grp, p>0.05 No change The length of time in normal sinus rhythm after occlusion was sig. longer, VF incidence was sig. higher, and the size of the ischemic zone was sig. larger when no Aspirin added during ischemia among the FO group. The length of time in normal sinus rhythm after occlusion was sig. longer, VF incidence was sig. higher, and the size of the ischemic zone was sig. larger when no Aspirin added during ischemia among the LO group.	Sex diff? No Age diff? No BW diff? ND

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Evidence Table 1: Whole Animal Studies

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Author, yr	Outcomes	Experimental Protocols	Results	Comment/ Biases/ Limitations
Kinoshita, 1994	VF (%) VT (%) AS VPB	Ischemia model: 3-hr coronary ligation Digitalis-induced arrhythmia model: Digoxin (0.025 mg/kg/min) was administrated intravenously over a 60 sec period to 5 dogs in each group immediately after ischemia	Ctrl: Standard diet [EPA vs. Ctrl] Ventricular extra beats (VEBs) in ischemia: n= 10 vs. 10, p<0.05 Decrease (sig.) -44% Estimated VF (%) in ischemia: n= 10 vs. 10, p>0.05 No change 2/10 vs. 2/10 Reported AS in ischemia: n= 10 vs. 10, p<0.05 Decrease (sig.) -55% Estimated Time in developing digitalis-induced VT or VF: n= 5 vs. 5, Increased >25 min vs. 10-15 min Reported	Sex diff? ND Age diff? ND BW diff? ND
Lo, 1991	VT (%) VPB	Normal conditions.	No events of VT or VPB were observed when infusing control buffer, ALA=1, 5, or 10 mg/kg (n= 8, same dogs for all groups) [ALA vs. Ctrl buffer n=8, same dogs for all groups] VT (%) ALA=20, or 30 mg/kg, p>0.05 Increased 13%, or 38% respectively Reported ALA=60 mg//kg, p=0.013 Increase (sig.) 63% Reported VPB (%) ALA=20 mg/kg, p>0.05 Increased 25% Reported ALA=30 mg/kg, p=0.003 Increase (sig.) 75% ALA=60 mg/kg, p=0.0007 Increase (sig.) 88% Reported All ventricular arrhythmia occurred within 3 seconds after injection and recovered spontaneously within 5 minutes. A significant depression of myocardial contractility (the change in maximal left ventricular dp/dt during systole) was noted at a dose of 5 mg/kg. When the dose of ALA was increased, a more prominent myocardial depression was observed. In contract, and injection of the control buffer solution did not alter left ventricular dp/dt.	Sex diff? No Age diff? ND BW diff? ND Injections of ALA invariably result in acute pulmonary edema within 5 minutes (unpublished observation). Thus, whether or not the observed CVD effect was due to the direct effect of ALA was questionable. Possibly cumulative effect should be considered because various testing doses were repeatedly given in each dog.
McLennan, 1996	VF (%) AS	Ischemia model	Ctrl: Olive oil AS (results in figure) [EPADHA vs. Ctrl] n= "n=10", p<0.02 Decrease (sig.) [EPA vs. Ctrl] n=ND, p>0.05 No change [DHA vs. Ctrl] n= "n=10", p<0.02 Decrease (sig.) VF (%) [EPADHA vs. Ctrl] n=ND p<0.01 Inhibitory effects 10%/80% Reported [EPA vs. Ctrl] n=ND, p>0.05 No change 70%/80% Reported [DHA vs. Ctrl] n=ND ", p<0.03 Inhibitory effects 20%/80% Reported	Sex diff? No Age diff? ND BW diff? ND No statistics were performed for these comparisons in the original study.

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Evidence Table 1: Whole Animal Studies

Part 2

Author, yr	Outcomes	Experimental Protocols	Results	Comment/ Biases/ Limitations
McLennan, 1995	VPB VT (%) VF (%) AS Deaths	Myocardial ischemia and reperfusion models: 15-min ischemia; 10-min reperfusion. 5-min ischemia; 10-min reperfusion.	15-min ischemia; 10-min reperfusion model: VPB [CAN vs. SSO in ischemia] n= 16 vs. 14, No change -13% Estimated [SBO vs. SSO in ischemia] n= 13 vs. 14, No change -14% Estimated [CAN vs. SSO in reperfusion] n= 13 vs. 13, No change -43% Estimated [SBO vs. SSO in reperfusion] n= 11 vs. 13, No change -2% Estimated VT (%) [CAN vs. SSO in ischemia] n= 16 vs. 14, No change 75%/93% Reported [SBO vs. SSO in ischemia] n= 13 vs. 14, No change 62%/93% Reported [CAN vs. SSO in reperfusion] n= 13 vs. 13, No change 31%/69% Reported [SBO vs. SSO in reperfusion] n= 11 vs. 13, No change 3%/69% Reported VF (%) [CAN vs. SSO in ischemia] n= 16 vs. 14, No change 43%/43% Reported [SBO vs. SSO in ischemia] n= 13 vs. 14, No change 38%/43% Reported [CAN vs. SSO in reperfusion] n= 13 vs. 13, Decrease (sig.) 0%/23% Reported [SBO vs. SSO in reperfusion] n= 11 vs. 13, No change 27%/23% Reported VF Deaths [CAN vs. SSO in ischemia] n= 16 vs. 14, Increased 19%/7% Reported [SBO vs. SSO in ischemia] n= 13 vs. 14, Increased 15%/7% Reported [CAN vs. SSO in reperfusion] n= 13 vs. 13, Decrease (sig.) 0%/8% Reported [SBO vs. SSO in reperfusion] n= 11 vs. 13, Decrease (sig.) 0%/8% Reported AS [CAN vs. SSO in ischemia] n= 16 vs. 14, No change -11% Estimated [SBO vs. SSO in ischemia] n= 13 vs. 14, No change -18% Estimated [CAN vs. SSO in reperfusion] n= 13 vs. 13, No change -64% Estimated [SBO vs. SSO in reperfusion] n= 11 vs. 13, No change -12% Estimated 5-min ischemia; 10-min reperfusion model: VPB [CAN vs. SSO in reperfusion] n= 10 vs. 10, p>0.05 No change [SBO vs. SSO in reperfusion] n= 10 vs. 10, p>0.05 No change VT (%) [CAN vs. SSO in reperfusion] n= 10 vs. 10, No change 70%/70% Reported [SBO vs. SSO in reperfusion] n= 10 vs. 10, No change 90%/70% Reported VF (%) [CAN vs. SSO in reperfusion] n= 10 vs. 10, No change 10%/50% Reported [SBO vs. SSO in reperfusion] n= 10 vs. 10, No change 50%/50% Reported VF Deaths [CAN vs. SSO in reperfusion] n= 10 vs. 10, 0%/20% Reported	Sex diff? No Age diff? No BW diff? ND

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Evidence Table 1: Whole Animal Studies

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Author, yr	Outcomes	Experimental Protocols	Results	Comment/ Biases/ Limitations
McLennan, Bridle, 1993	VFT VF (%)	Ischemia was induced by 15-min coronary artery occlusion. Isoproterenol (ISO) was injected in 0.5 ug/kg BW/min. Arrhythmias were induced by electrical stimulation protocol in control, 5 min after ischemia, and 30 min after restoration of coronary blood flow during the infusion of isoproterenol.	Ctrl: SF/SSO (8% sheep perirenal fat+2% sunflower-seed oil) [SF/FO vs. Ctrl] VF (%) in control condition: n=10 vs. 9, No Change 60%/60% Reported VF (%) in ischemia: n=10 vs. 9, No Change 100%/100% Reported VF (%) in ISO (0.5 ug/kg body wt/min): n= 10 vs. 9, p<0.05 Decrease (sig.) 3/10 vs. 7/9 Reported. The other 2 out of the 10 animal in FO group developed VT. VF (%) in ISO (2.0 ug/kg body wt/min): n=: 10 vs. 9, p=0.033 Decrease (sig.) 5/10 vs. 9/9 Reported Among susceptible animals: VFT in control condition: n= 6 vs. 6, p<0.05 Increase (sig.) +133% Estimated VFT in ischemia: n= 10 vs. 10, p<0.05 Increase (sig.) +79% Estimated VFT in ISO (0.5 ug/kg body wt/min): n= 5 (VF+VT) vs. 7, p<0.05 Increased +55% Estimated VFT in ISO (2.0 ug/kg body wt/min): n= 5 vs. 9, p>0.05 Increased +75% Estimated	Sex diff? No Age diff? ND BW diff? No

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Evidence Table 1: Whole Animal Studies

Part 2

Author, yr	Outcomes	Experimental Protocols	Results	Comment/ Biases/ Limitations
McLennan, 1993	Time in normal sinus rhythm VPB VT (%) VF (%) AS Deaths	Myocardial ischemia and reperfusion models: 15-min ischemia; 5-min reperfusion. 5-min ischemia; 5-min reperfusion.	15-min ischemia; 5-min reperfusion model: VPB [FO vs. SSO in ischemia] n= 14 vs. 13, No change -10% Estimated [FO vs. SSO in reperfusion] n= 14 vs. 12, No change -31% Estimated VT (%) [FO vs. SSO in ischemia] n= 14 vs. 13, Decrease 35%/92% Reported [FO vs. SSO in reperfusion] n= 14 vs. 12, p<0.05 Decrease (sig.) 21%/67% Reported VF (%) [FO vs. SSO in ischemia] n= 14 vs. 13, Decrease (sig.) 0%/38% Reported [FO vs. SSO in reperfusion] n= 14 vs. 12, p>0.05 No Change, 0%/8% Reported Time in sinus rhythm [FO vs. SSO in ischemia] n= 14 vs. 13, No change +12% [FO vs. SSO in reperfusion] n= 14 vs. 12, p>0.05 No Change Deaths [FO vs. SSO in ischemia] n= 14 vs. 13, Decrease (sig.) 0%/8% Reported [FO vs. SSO in reperfusion] n= 14 vs. 12, No change 0%/0% Reported AS [FO vs. SSO in ischemia] n= 14 vs. 13, , p<0.05 Decrease (sig.) -41% Estimated [FO vs. SSO in reperfusion] n= 14 vs. 12, p<0.05 Decrease (sig.) –63% Estimated 5-min ischemia; 5-min reperfusion model VPB [FO vs. SSO in reperfusion n= 10 vs. 12, No change -27% Estimated VT (%) [FO vs. SSO in reperfusion] n= 10 vs. 12, No change 60%/80% Reported VF (%) [FO vs. SSO in reperfusion] n= 10 vs. 12, Decrease 10%/25% Reported Time in sinus rhythm [FO vs. SSO in reperfusion] n= 10 vs. 12, No change +16% Estimated Deaths [FO vs. SSO in reperfusion] n= 10 vs. 12, Decrease (sig.) 0%/8% Reported AS [FO vs. SSO in reperfusion] n= 10 vs. 12, Decrease -48% Estimated	Age diff? No BW diff? No No statistics were performed for FO vs. SSO comparisons in the original study.

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Evidence Table 1: Whole Animal Studies

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Author, yr	Outcomes	Experimental Protocols	Results	Comment/ Biases/ Limitations
McLennan, 1992	VFT VF (%) Deaths	Ischemia was induced by 15-min coronary artery occlusion. Isoproterenol (ISO) was injected in 0.5 ug/kg BW/min. Arrhythmias were induced by electrical stimulation protocol in control, 5 min after ischemia, and 30 min after restoration of coronary blood flow during the infusion of isoproterenol.	VF (%) [TFO vs. SSO in control condition] n= 16 vs. 13, No change 10/16 vs. 8/13 Estimated from graph [TFO vs. SSO in ischemia] n= 16 vs. 13 No change 12/16 vs. 8/13 Estimated from graph [TFO vs. SSO in ISO] n= 16 vs. 13 No change 7/16 vs. 10/13 Estimated from graph In TFO fed animals, 80% of VF episodes were of less than 5 seconds' duration compared with only 25% of SSO (p=0.054 n.s.) animals. VFT in susceptible marmosets (results in figure) [TFO vs. SSO in control condition] n= 10 vs. 8, p>0.05 No change [TFO vs. SSO in ischemia] n= 12 vs. 8, p>0.05 No change [TFO vs. SSO in ISO] n= 7 vs. 10, >0.05 No change Total VF Deaths, combining the Deaths in control condition, ischemia, and isoproterenol models. [TFO vs. SSO] n= 16 vs. 13, Decrease (sig.) 0/16 vs. 3/13 Reported	Sex diff? ND Age diff? No BW diff? No Isoproterenal induced a sig. increase in the proportion of inducible animals having sustained episodes of VF in all dietary groups; as well as a sig. proportion of animals suffering fatal VF and cardiac arrest compared to the control condition.
McLennan, 1990	Time in normal sinus rhythm VPB VT (%) VF (%) AS Deaths	Myocardial ischemia and reperfusion models: 15-min ischemia; 10-min reperfusion.	VPB [SF/TFO vs. SF/SSO in ischemia] n= 7 vs. 7, p>0.05 No change [SF/TFO vs. SF/SSO in perfusion] n= 7 vs. 7, p<0.05 Decrease (sig.) -24% Estimated VT (%) [SF/TFO vs. SF/SSO in ischemia] n= 7 vs. 7, p>0.05 No change 57%/57% Reported [SF/TFO vs. SF/SSO in perfusion] n= 7 vs. 7, p>0.05 No change 71%/86% Reported VF (%) [SF/TFO vs. SF/SSO in ischemia] n= 7 vs. 7, p>0.05 No change 14%/29% Reported [SF/TFO vs. SF/SSO in perfusion] n= 7 vs. 7, p>0.05 No change 29%/29% Reported Time in sinus rhythm [SF/TFO vs. SF/SSO in ischemia] n= 7 vs. 7, p>0.05 No change [SF/TFO vs. SF/SSO in perfusion] n= 7 vs. 7, p>0.05 No change VT/VF Deaths [SF/TFO vs. SF/SSO in ischemia] n= 7 vs. 7, No change 0%/0% Reported [SF/TFO vs. SF/SSO in perfusion] n= 7 vs. 7, No change 0%/0% Reported AS (results in figure [SF/TFO vs. SF/SSO in ischemia] n= 7 vs. 7, p>0.05 No change [SF/TFO vs. SF/SSO in perfusion] n= 7 vs. 7, p>0.05 No change	Sex diff? No Age diff? No BW diff? Yes SSO group had significant lower body weight after 18 months

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Evidence Table 1: Whole Animal Studies

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Author, yr	Outcomes	Experimental Protocols	Results	Comment/ Biases/ Limitations
McLennan PL 1988	VT (%) VF (%) AS Ischemic region (%)	Myocardial ischemia and reperfusion models: 15-min ischemia, followed by reperfusion.	VT (%) [TFO vs. SSO in ischemia] n= 10 vs. 10, p<0.01 Decrease (sig.) 37% vs. 77%* Reported [TFO vs. SSO in reperfusion] n= 10 vs. 10, p<0.05 Decrease (sig.) 50%* vs. 80%* Estimated VF (%) [TFO vs. SSO in ischemia] n= 10 vs. 10, p<0.01 Decrease (sig.) 0% vs. 10%* Reported [TFO vs. SSO in reperfusion] n= 10 vs. 10, p<0.05 Decrease (sig.) 0% vs. 30% Estimated AS (results in figure) [TFO vs. SSO in ischemia] n= 10 vs. 10, p>0.05 No change [TFO vs. SSO in reperfusion] n= 10 vs. 10, p<0.05 Decrease (sig) -44% Estimated IS [TFO vs. SSO] n= 10 vs. 10, No change +7% Estimated No animal died in TFO and SSO groups.	Sex diff? No Age diff? No BW diff? Yes SSO=512 gm; TFO=566 gm
Oskarsson, 1993	SIZ (%)	Myocardial ischemia and reperfusion models: 90-min ischemia; 30-min reperfusion.	Ctrl: No fish oil Rx [MaxEPA vs. Ctrl] Myocardial infarct size (%): n= 10 vs. 12, p<0.05 Decrease (sig.) 13%/29% Reported Amount of myocardium at risk for severe ischemia (%): n= 10 vs. 12, p>0.05 No change 41.2%/39.3% Reported	Sex diff? ND Age diff? ND BW diff? No
Otsuji, 1993	Deaths Infracted area Size of the area at risk (%)	Coronary artery occlusion (ischemia model)	Ctrl: Standard dog chow [EPA-ester vs. Ctrl] VF Deaths: n= 10 vs. 15, p<0.05 Decrease (sig.) 0%/33% Reported Ultimate size of the infarcted area: n= 10 vs. 10, p<0.01 Decrease (sig.) 17.6%/29.2% Reported Size of the area at risk (%): n= 10 vs. 10, p>0.05 No change	Sex diff? ND Age diff? ND BW diff? ND

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Author, yr	Outcomes	Experimental Protocols	Results	Comment/ Biases/ Limitations
Pepe, 1996	VPB VT (%) VF (%) VFT	Myocardial ischemia and reperfusion models: 15-min ischemia; 10-min reperfusion. Electrical Stimulation (independent of myocardial ischemia model)	[FO vs. SAT] Ischemia-reperfusion model: VPB during ischemia: n= 20 vs. 20, p<0.05 Decrease (sig.) -73% Estimated VT (%) after ischemia-reperfution: n= 20 vs. 20, p<0.05 Decrease (sig.) 10%/70% Reported VF (%) after ischemia-reperfution: n= 20 vs. 20, p<0.05 Decrease (sig.) 0%/80% Reported Electrical Stinulation: The threshold current for VF induction was reduced in all dietary groups in ischemia but remained significantly higher in the mackerel-oil-fed group than in the saturated-fat-fed group (n=10 per group).	Sex diff? No Age diff? No BW diff? ND
Yang, 1993	VF (%) or VF (%)	Myocardial ischemia and reperfusion models: 15-min ischemia; 10-min reperfusion.	[FO vs. Butter] VT (%) or VF (%): n= 8 vs. 9, p<0.05 Decrease (sig.) 3/8 vs. 7/9 Reported	Sex diff? No Age diff? ND BW diff? ND

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Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies

Part 1

Author, yr	Country Funding	Species Stage Sex	Exposure Duration (weeks)	Group [Sample Size]	Total Fat (omega-3 fatty acids)	Unit	SFA	MUFA	PUFA	ALA	EPA	DHA	Other omega-3 fatty acids
Benediktsdottir, 1988	Iceland U	Rats Adult Male	16	Corn oil (CO) [ND] Cod-liver oil (CLO) [ND]	10 %w 10 %w	% total fatty acids	14.5 22.0	24.5 47.0	57.8 27.2	ND ND	0.0 6.9	0.0 7.2	0.0[3] 0.9[3]
Black, 1989	Canada G	Rats Adult Male	4	STD [6] STD+FO [6]	 0.5ml/kg/day	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND	
Chemla, 1995	France G	Rats Adult Male	4	N-3 [15] N-6 [15]	15%w 15%w	%TFA	20.0 19.8	57.7 58.9	11.4 20.7	0.8 0.5	4.3 0.0	4.1 0.0	
Chen, 1994	Taiwan G	Rabbits Adult Male	2	High cholesterol (HC) [11-15] HC+FO [11-12]	40 %kcal 40 %kcal (10 %kcal from fish oil)	% w/w	ND ND	ND ND	ND ND	ND ND	ND 30.2	ND 21.5	
Croset, 1989a	USA G[1]	Mouse Weanling Male	2	STD [10] STD+0.4 %w/w DHAe[10] STD+0.8 %w/w DHAe[10] STD+ 4%w/w DHAe [10]	0 %w 10 %w/w 10 %w/w 10 %w /w	Mol%	11.8 11.7 11.5 5.3	31.7 28.8 25.7 9.3	56.1 59.1 61.6 85.3	0.0 0.0 0.0 0.0	ND ND ND ND	0.0 0.1 0.2 0.8	
Croset, 1989b	USA G	Mouse Weanling Male	2	OO+ALA e [6] OO+EPA e [6] OO+DHA e [6]	1.5+0.5%w 1.5+0.5%w 1.5+0.5%w	Mol%	27.6 30.3 27.5	44.5 49.8 47.3	27.9 19.9 25.1	20.5 1.0 0.5	0.2 8.1 0.9	0.1 1.9 16.5	
Demaison, 1993	France G	Rats Weanling Male	8	SF [32] LIN [29]	100g/Kg 100g/kg	% TFA	11.8 8.7	16.2 20.3	71.7 71.0	0.2 53.5	ND ND	ND ND	
Gillis, 1992	Canada G	Rabbits Weanling ND	6	SAF (9) FO (9)	10%w 10%w	%w	9.6 23.5	13.1 29.2	77.3 47.3	0.0 1.4	0.0 26.5	0.0 8.6	0.0[3] 2.3[3]

Appendix C

Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies

Part 1

Author, yr	Country Funding	Species Stage Sex	Exposure Duration (weeks)	Group [Sample Size]	Total Fat (omega-3 fatty acids)	Unit	SFA	MUFA	PUFA	ALA	EPA	DHA	Other omega-3 fatty acids
Gudmundsdottir, 1991	Iceland U	Rats Adult Male	20	CO [5] CLO (4)	10%w 10%w	%w	13.6 18.1	24.6 51.0	58.6 27.7	2.6 0.0	0.0 7.1	0.0 8.1	
		Rats Aged Male	88	CO [5] CLO (4)	10%w 10%w	%w	13.6 18.1	24.6 51.0	58.6 27.7	2.6 0.0	0.0 7.1	0.0 8.1	
Heard, 1992	USA U	Rats Adult Male	4	SAF [18] MenO+SAF [18]	20%w 19.5%+0.5 %w	ND	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND	
Honen, 2002	Austra-lia G	Rats Adult Male	3	Canola oil (6) FO(6) G	3ml/d 3ml/d	% TFA	6.2 1.7	60.0 15.8	33.8 77.7	12.1 0.5	0 48.0	0 26.2	
Karmazyn, 1987	Canada G	Rats Weanling Male/ Female	12	STD [14] STD+Cod liver oil (CLO) [14]	10%w	ND	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND	
Kinoshita, 1994	Japan U	Dogs Adult ND	8	STD (15) STD+EPAe (15)	100mg/kg	mg/kg/d	ND ND	ND ND	ND ND	ND ND	ND 100	ND ND	
Ku, 1997	Japan G	Rats Aged Female	12	HC (5) HC+EPA (5) HC+DHA (5)	5.1%w 5.1%w (300mg/kg) 5.1%w (300mg/kg)	ND	ND ND ND	ND ND ND	ND ND ND	ND ND ND	ND ND ND	ND ND ND	
Lamers, 1988	Neth.; Italy G	Pigs Weanling Male/ Female	8	LARD [8] FO +LARD [8]	9%w 4.5% +4.5%w	%TFA	36 32	46 40	15 11	1 1	0 8	0 5	
Laustiola, 1986	Finland U	Rats Weanling Male	16	STD [20] STD+CLO [33]	10%na	% TFA	26.2 22.8	23.6 46.5	49.7 28.6	5.3 1.7	1.3 6.4	2.7 8.2	0.2[3] 2.7[4] 0.7[3] 8.2[4]

Appendix C

Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies

Part 1

Author, yr	Country Funding	Species Stage Sex	Exposure Duration (weeks)	Group [Sample Size]	Total Fat (omega-3 fatty acids)	Unit	SFA	MUFA	PUFA	ALA	EPA	DHA	Other omega-3 fatty acids
Leifert, 2000a	Australia G	Rats Young Adult Male	3	LARD [6-8] FO [6-8] G5	29% E (74kJ fat/d) 29% E (74kJ fat/d)	% w	58.0 27.3	39.4 28.2	2.6 44.6	0.7 1.1	0.1 24.3	0.0 12.1	0.0[3] 2.3[3]
Leifert, 2001	Australia I+NP	Rats Adult Male	3	SF (6) FO (6)	17%w (10%w) 17%w (10%w)	%w	36.4 18.6	55.1 44.0	8.5 37.4	1.2 0.9	0 17.8	0 8.9	0.0[3] 1.7[3]
Maixent, 1999	France G+NPI	Rats Adult Male	8	STD [11] STD+FO [10]	0.5g of oil/kg	mg/g of oil	ND ND	ND ND	ND ND	ND ND	ND **180**	ND **120**	
Minaro-vic, 1997	Slovak G	Rats Young Adult Male	2	HF [10] FO [10]	300g/kg 100g/kg	% w	47.0 13.0	39.7 29.4	13.3 57.6	ND ND	ND ND	ND ND	
Pepe, 1999	USA U	Rats Young Adult Male	6	N-6 (6) FO (5)	15.6% w (11.7%w) 15.6%w (11.7%w)	ND	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND
Reig, 1993	Spain U	Rats Young Adult Male	5	HF (20) HF+FO (20)	37%w 31%+6%w	%TFA	36.7 30.0	40.0 33.0	19.4 37.1	2.2 3.4	0.0 4.6	0.0 3.4	0.0[3] 1.0[3]
Swan-son, 1989	USA G	Mouse Weanling Male	2	SAF+CO (9) SAF+MenO (9)	12%w (2%+10%w) 12%w (2%+10%w)	%w	14.5 28.5	24.2 26.1	60.9 44.7	1.0 1.8	0.0 12.9	0.0 9.1	0.0[3] 2.0[3]
Taffet, 1993	USA G	Rats Young Adult Female	3	CO [11] CO+MenO [12]	20%w 3%+17%w	Mol %	14.3 39.9	26.3 28.3	59.3 31.9	0.0 1.3	0.0 16.5	ND ND	

Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies
Part 2

Author, yr	Outcome Category	Cell or Tissue	Expt Condition	Agent	Change in Membrane Composition	Results (n=cells)
Benedikt-sdottir, 1988	IPIM	Sarcolemma	Ambient	None	Yes	CLO vs. CO - NC in Na^+K^+ATPase (n=ND; p>0.05)
Black, 1989	IPIM	Sarcoplasmic reticulum	Ambient	None	ND	STD+FO vs. STD - NC in sarcoplasmic reticulum calcium transport activity (n=6;p>0.05)
Chemla, 1995	CP	Myocardium	Ambient	None	ND	N-3 vs. N-6 - NC in force-velocity relationship characteristics (n=15; p>0.05)
Chen, 1994	IPIM	Myocardial mitochondria	Sham ischemic	None	ND	HC+FO vs. HC - NC in mitochondrial calcium concentrations (n=5/group; p>0.05)
			Short-term Ischemia (Occl-10min Rep-1hr) Long-term Ischemia (Occl-1hr Rep-4hr)	None	ND	HC+FO vs. HC - NC in mitochondrial calcium concentrations after short or long term ischemia (n=11/group; p>0.05)
Croset, 1989a	IPIM[1]	SR vessicles[2]	Ambient	None	Yes	All DHA diets vs. STD - NC in maximum velocity of SR Ca2+, Mg2+-ATPase with incremental levels of DHA (n=10/group; p>0.05)
		Myocardial mitochodria	Ambient	0.15 uM oligomycin	Yes	STD+0.4%DHAe. vs. STD - Increased maximum velocity of mitochondrial oligomycin-sensitive ATPase (%=ND)(n=10/grp, p<0.02) STD+0.8%DHAe. vs. STD - Increased maximum velocity of mitochondrial oligomycin-sensitive ATPase (%=ND)(n=10/grp, p<0.05) STD+4%DHAe. vs. STD - NC in maximum velocity of mitochondrial oligomycin-sensitive ATPase (n=10/grp, p>0.05)

Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies
Part 2

Author, yr	Outcome Category	Cell or Tissue	Expt Condition	Agent	Change in Membrane Composition	Results (n=cells)
Croset, 1989b	IPIM	SR vesicles	Ambient	None	Yes	OO+ALAe. vs. Safflower oil - Decreased Ca^{2+} transport measured a maximum rate of Ca^{2+} accumulation in cardiac SR vesicles (%=ND) (n=3; p<0.05) OO+EPAe. vs. Safflower oil - Decreased Ca^{2+} transport measured a maximum rate of Ca^{2+} accumulation in cardiac SR vesicles (%=ND) (n=3; p<0.04) OO+DHAe. vs. O Safflower oil - Decreased Ca^{2+} transport measured a maximum rate of Ca^{2+} accumulation in cardiac SR vesicles (%=ND) (n=3; p<0.03)
						OO+ALAe. vs. OO+EPAe. - Decreased Ca^{2+} transport measured a maximum rate of Ca^{2+} accumulation in cardiac SR vesicles (%=ND) (n=3; p=ND) OO+ALAe. vs. OO+DHAe. - Decreased Ca^{2+} transport measured a maximum rate of Ca^{2+} accumulation in cardiac SR vesicles (%=ND) (n=3; p=ND) OO+EPAe. vs. OO+DHAe. - Decreased Ca^{2+} transport measured a maximum rate of Ca^{2+} accumulation in cardiac SR vesicles (%=ND) (n=3; p=ND) OO+EPAe. vs. OO+SA - NC in maximum specific activity (V_{max}) and affinity for Ca^{2+} and ATP of $Ca^{2+}Mg^{2+}$ATPase associated with CA2+ uptake (n=3;p>0.05) OO+DHAe. vs. OO+SA - NC in maximum specific activity (V_{max}) and affinity for Ca^{2+} and ATP of $Ca^{2+}Mg^{2+}$ATPase associated with CA2+ uptake (n=3;p>0.05)
Demaison, 1993	CP	Isolated Working heart	Ambient (Perfusion)	None	Yes	LIN vs. SF - NC in contractility (n=29-32; p>0.05)

Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies
Part 2

Author, yr	Outcome Category	Cell or Tissue	Expt Condition	Agent	Change in Membrane Composition	Results (n=cells)
Gillis, 1992	BEP	SR vesicles	Ambient	None	Yes	FO vs. SAF - NC in mean baseline QRS (n=9; p>0.05) - NC in mean baseline QT interval duration (n=9; p>0.05)
			Electrical	None	Yes	FO vs. SAF - NC in mean baseline left ventricular effective refractory period (VERP) (n=9; p>0.05) - NC in mean baseline epicardial MAP duration (n=9; p>0.05) - NC in mean baseline endocardial MAP duration (n=9; p>0.05) - NC in strength - interval relations (mean threshold current at each coupling interval) (n=9; p>0.05)
			Electrical	None	Yes	FO vs. SAF - NC in mean diastolic threshold (n=9; p>0.05) - NC in absolute refractory period (n=9; p>0.05) - NC in relative refractory period (n=9; p>0.05)
Gudmundsdottir, 1991	ICH	Sarcolemma	Ambient	NIT	Yes	CLO vs. CO at 20 weeks - NC in the affinity ($1/K_d$) and binding (B_{max}) of slow Ca2+ channels for [3H NIT] (n=4-5;p>0.05) CLO vs. CO at 88 weeks - Decreased the affinity (1/K) and binding (B_{max})of slow Ca2+ channels for [3H NIT] (n=4-5;p<0.05)
Heard, 1992	CP	Atrial tissue	Ambient	Saline	ND	FO+SAF vs. SAF - NC in force of contraction indexed to body weight (FOC/BW) (n=6; p>0.05) - NC in maximum rate of rise of contraction (dF/dt) (n=6; p>0.05) - NC in maximum rate of relaxation (-dF/dt) (n=6; p>0.05) - NC in atrial rate (beats/min) (n=6; p>0.05)
	CP	Atrial tissue	Ambient	LPS (20mg/kg)	ND	FO+SAF vs. SAF - Increased force of contraction indexed to body weight (FOC/BW) (n=11; p<0.05) - Increased maximum rate of rise of contraction (dF/dt) (n=11; p<0.05) - Increased maximum rate of relaxation (-dF/dt) (n=11; p<0.05) - Decreased atrial rate (beats/min) (n=ND; p<0.05)

Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies
Part 2

Author, yr	Outcome Category	Cell or Tissue	Expt Condition	Agent	Change in Membrane Composition	Results (n=cells)
	CP	Atrial tissue	Ambient	ISO (0.1, 0.5, 1.0 and 5.0uM)	ND	FO+SAF vs. SAF - NC in atrial force of contraction indexed to body weight (FOC/BW) as a function of Iso concentration (n=ND; p>0.05)
Honen, 2002	IPIM	Atrial myocytes	Ambient	None	Yes	FO vs. RO - NC in mean area of Ca2+ sparks n=5; p>0.05) - NC in mean duration of Ca2+ sparks (n=5; p>0.05) - NC in mean frequency of Ca2+sparks (n=5; p>0.05) - Increased the proportion of "ideal' sparks (rapid rise and exponential fall by 9.1% (n=5; p<0.05) - NC in proportion of "very slow rise and fall sparks" (n=5; p>0.05) - Decreased the proportion of "marked step/plateau in the decay phase" sparks by 63% (n=5; p<0.05)
Karma-zyn, 1987	IPIM	Ventricular myocytes	Ischemia-Reperfusion	None	Yes	STD+CLO vs STD - Time dependent (10-80mins) increase in Ca^{2+} uptake by 135-159% (n=5-9; p<0.01) - NC in Ca^{2+} efflux (n=5-9; p>0.05)
	BEP	Ventricular myocytes	Ischemia-Reperfusion	None	Yes	STD+CLO vs STD - NC in developed or resting tension (n=5-9; p>0.05)
Kinoshita, 1994	IPIM	Myocardial microsomal vesicles	Ambient (non-infarct)	None	Yes	STD+EPAe. vs. STD - Increased Ca^{2+}-Mg^{2+}ATPase V_{max} by 48% (n=6; p<0.01) - NC in Km (n=6; p>0.05)
		Myocardial microsomal vesicles	Ischemia	None	Yes	STD+EPAe. vs. STD - Increased Ca^{2+}-Mg^{2+}ATPase V_{max} by 45% (n=6; p<0.01) - NC in Km (n=6; p>0.05)
		Myocardial microsomal vesicles	Ambient (non-infarct)	Oua	Yes	STD+EPAe. vs. STD - NC in Na^{2+}-K^{2+}ATPase V_{max} (n=6; p>0.01) - NC in the amount of ouabain needed to induce 50% inhibition (IC_{50}) of Na^{2+}-K^{2+}ATPase activity.(n=6; p>0.05)
		Myocardial microsomal vesicles	Ischemia	Oua	Yes	STD+EPAe. vs. STD - NC in Na^{2+}-K^{2+}ATPase V_{max} (n=6; p>0.01) - NC in the amount of ouabain needed to induce 50% inhibition (IC_{50}) of Na^{2+}-K^{2+}ATPase activity.(n=6; p>0.05)

Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies
Part 2

Author, yr	Outcome Category	Cell or Tissue	Expt Condition	Agent	Change in Membrane Composition	Results (n=cells)
Ku, 1997	CP	Isolated Heart	Ambient	None	Yes	HC+EPA vs. HC - NC in recovery of heart rate (n=5; p>0.05)
			Ambient	None	Yes	HC+DHA vs. HC - NC in recovery of heart rate (n=5; p>0.05)
			Ambient	None	Yes	HC+DHA vs HC+EPA - NC in recovery of heart rate (n=5; p>0.05)
Lamers, 1988	IPIM	Sarcolemma	Ambient	Ca^{2+}	Yes	FO+LARD vs LARD - Increased Ca^{2+} pumping ATPase activity by 68% (n= 6; p< 0.05)
			Ischemia (5 min) Reperfusion (10 min)	Ca^{2+}	Yes	FO+LARD vs LARD - Increased Ca^{2+} pumping ATPase activity by 43% (n=6; p< 0.05)
Laustiola, 1986	CP	Atrial myocytes	High O2	None	Yes	STD+CLO vs STD - Decreased contraction amplitude by 25% (n=7-11; p<0.001) - Decreased heart rate by 24% (n=7-11; p<0.001)
			High O2	NA($1x10^{-6}$/90sec)	Yes	STD+CLO vs STD - NC in contraction amplitude (n=4-11; p>0.05) - NC in heart rate (n=4-11; p>0.05)
			Hypoxia	NA($1x10^{-6}$/90sec)	Yes	STD+CLO vs STD - Decreased contraction amplitude by 58% (n=4-11; p<0.001) - Decreased heart rate by 13% (n=4-11; p<0.001)
			Reoxy O2 5min	NA ($1x10^{-6}$/90sec)	Yes	STD+CLO vs STD - NC in contraction amplitude (n=4-11; p>0.05) - NC in heart rate (n=4-11; p>0.05)

Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies
Part 2

Author, yr	Outcome Category	Cell or Tissue	Expt Condition	Agent	Change in Membrane Composition	Results (n=cells)
Leifert, 2000a	CP	Ventricular myocytes	Ambient		Yes	FO vs. LARD - NC in resting or diastolic cell length (voltage required to stimulate 90% of cells to contract) (n=29-36; p>0.05) - NC in systolic cell length (peak cell shortening during steady state) (n=29-36; p>0.05) - NC in percent cell length (systolic-diastolic/diastolic*100) (n=29-36; p>0.05) - NC in post-rest potentiation (post rest contraction length/steady state contraction length*100) (n=29-36; p>0.05)
			Ambient	ISO (0.01-3uM/3min)	Yes	FO vs. LARD - Decrease and delay in the development of ISO induced asynchronous contractile activity (n=6 animals/gr; p<0.05) - EC_{50} values were 892 ± 130nM and 347 ± 91 nM for FO and LARD, respectively.
			Ambient	FRGS (2.3mM purine; 7mU/ml xanthine oxidase/20mins)	Yes	FO vs. LARD - Decreased development of FRGS induced asynchronous contractions over the entire time course (3-20mins) (n=6-9 animals/gp; p<0.01) - Increased the time taken until 50% of cardiomyocytes contracted in an asynchronous manner (30%) (n=6-9animals/gp; p<0.01)
			Ambient	None	Yes	FO vs. LARD - NC in voltage dependence of Na^+ current activation parameters G_{max}; V_{50}; E_{rev} or K (n=28; p>0.05) - NC in voltage dependence of Na^+ current inactivation parameters I_{max} and K (n=28; p>0.05) - More negative V_{50} for the voltage dependence of Na^+ current inactivation (n=28; p<0.05)
			Ambient	None	Yes	FO vs. LARD - NC in I_{to} current activation parameters I_{max}; V_{50} or K (n=17-28; p>0.05) - NC in I_{to} current inactivation parameters I_{max}; V_{50} or K (n=17-28; p>0.05)

See abbreviations in List of Acronyms, Abbreviations and Parameters

Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies
Part 2

Author, yr	Outcome Category	Cell or Tissue	Expt Condition	Agent	Change in Membrane Composition	Results (n=cells)
Leifert, 2001	CP	Cardio-myocytes	Ambient	ISO (0.1-3uM)	Yes	FO vs. SF - Decreased the time of onset of asynchronous contractile activity (%=ND)(n=6 animals; p<0.001) - No change in the number of asynchronously contracting myocytes (n= 29-32; *p>0.05*) - Prevented asynchronously contraction during Ca2+ transient measurements (n= 29-32; p<0.05)
	IPIM		Ambient	None	Yes	FO vs. SF - NC in SR Ca2+ transient under steady state conditions and after 30sec rest period (n=8/grp; p>0.05)
			Ambient	Caffeine (20mM) – induced SR Ca2+ release	Yes	FO vs. SF - NC in SR Ca2+ content (n=8/grp; p>0.05)
			Ambient	DBHQ (10uM/4m): SR Ca2+ ATPase inhibitor	Yes	FO vs. SF - NC in value of the peak Ca2+ transient (n=32; p>0.05) - Increased the time constant of decay (tau) of the Ca2+ transient (%=ND) (n=8/grp; p<0.05), indicating a more rapid Ca2+ efflux via sarcolemmal Ca2+ exchangers in the SF group.
			Ambient	None	Yes	FO vs. SF - Increased the time constant of the decay phase of the Ca2+ transient (%=ND) (n=8/grp; p<0.05) - NC in end-diastolic Ca2+ concentration (n=8/grp; p>0.05) - NC in systolic peak Ca2+ concentration (n=8/grp; p>0.05) - NC in developed Ca2+ concentration (systolic-end diastolic) (n=8/grp; p>0.05)

Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies
Part 2

Author, yr	Outcome Category	Cell or Tissue	Expt Condition	Agent	Change in Membrane Composition	Results (n=cells)
			Ambient	ISO (0.5uM): increase the cellular Ca2+ load	Yes	FO vs. SF - Increased the time constant of the decay phase of the Ca2+ transient by 13% (n=8/grp; p<0.01), indicating a more rapid Ca2+ efflux via SR and/or sarcolemmal Ca2+ exchangers in the SF group. - NC in end-diastolic Ca2+ concentration (n=8/grp; p>0.05) - NC in systolic peak Ca2+ concentration (n=8/grp; p>0.05) - NC in developed Ca2+ concentration (systolic-end diastolic) (n=8/grp; p>0.05)
Maixent, 1999	IPIM	Myocardial microsomal vesicles	Ambient	OUA (10^{-7} to 10^{-4}M)	Yes	STD+FO vs. STD - NC in ouabain-sensitive Na^+K^+ATPase activity (n=4; p>0.05) - NC in relative contribution of Na^+K^+ATPase a2 isoform (high affinity) - NC in relative contribution of Na^+K^+ATPase a1 isoform (low affinity) - NC in IC_{50} value of Na^+K^+ATPase a2 isoform (high affinity) - Lower IC_{50} value of Na^+K^+ATPase a1 isoform (low affinity)
Minarovic, 1997	ICU	Ventricular myocytes	Ambient	None	Yes	FO vs. HF - NC in rates of activation and the fast component of inactivation of the Ca^{2+} current (n=ND; p>0.05) - More negative half–inactivation potential (n=5-8;p<0.05) suggesting that these channels are less prone to inactivation - NC in voltage dependence of the peak I_{Ca} amplitude (n=ND; p>0.05)
			Ambient	Verapamil (ND)	Yes	FO vs. HF - No effect on the binding characteristics of the calcium channel blockers or the parameters of the I_{Ca}-V curves (n=ND; p>0.05)

Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies
Part 2

Author, yr	Outcome Category	Cell or Tissue	Expt Condition	Agent	Change in Membrane Composition	Results (n=cells)
			Ambient	Diltiazem: (ND)	Yes	FO vs. HF - No effect on the binding characteristics characteristics of the calcium channel blockers or the parameters of the I_{Ca}-V curves (n=ND; p>0.05)
Reig, 1993	BEP	Ventricular Tissue	Ambient	None	Yes	HF+FO vs. HF - Decreased ventricular refractory period by 14% (n=5 animals; *p<0.05*)
	CP	Ventricular Tissue	Ambient	None	Yes	HF+FO vs. HF - No change in proportion of animals with heart rate >750 beats/min by 50% (n=10animals; *p>0.05)*
Pepe, 1999	IPIM	Myocardial mitochondria	Ambient	None	Yes	FO vs. N-6 in young animals - NC in response of mitochondrial Ca2+concentration (n=5-6; p>0.05) FO vs. N-6 in aged animals - NC in response of Ca2+concentration (n=5-6; p>0.05) FO vs. FO (aged vs. young) - NC in response of Ca2+concentration (n=5-6; p>0.05)
			Ambient	Norepinephrine (10^{-7} M): ß-adrenergic receptor stimulation	Yes	FO vs. N-6 in young animals - Decreased response of Ca2+concentration by 32% (n=5-6; p<0.05) FO vs. N-6 in aged animals - Decreased response of Ca2+concentration by 35% (n=5-6; p<0.05) FO vs. FO (aged vs. young) - NC in response of Ca2+concentration (n=5-6; p>0.05)
			15-min low-flow ischemia, and 5-min reperfusion	None		FO vs. N-6 in young animals - Decreased response of Ca2+concentration (n=6; p<0.0001) FO vs. N-6 in aged animals - Decreased response of Ca2+concentration (n=6; p<0.05)

Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies
Part 2

Author, yr	Outcome Category	Cell or Tissue	Expt Condition	Agent	Change in Membrane Composition	Results (n=cells)
Swanson, 1989	IPIM	SR vesicles	Ambient	None	Yes	SAF+MenO vs SAF+CO - Decreased relative activity of Ca2+-Mg2+ ATPase activity by 86% (n= 3 pools of 3 hearts per replicate; p<0.05) SAF+MenO vs SAF+CO - Decreased the initial (0-5 min) calcium transport rate by 60% (n= 3 pools of 3 hearts per replicate; p<0.05) SAF+MenO vs SAF+CO - Decreased maximum sarcoplasmic reticulum calcium uptake by 62% (n= 3 pools of 3 hearts per replicate; p<0.01)
Taffet, 1993	IPIM	Ventricular SR vesicle	Ambient	None	Yes	CO+FO vs. CO - Decreased oxalate facilitated ATP dependent SR Ca2+ uptake by 30% (n=11-12; p<0.05)
			Ambient	Calcium 40uM +ATP 50um	Yes	CO+FO vs. CO - Decreased $Ca^{2+}Mg^{2+}$ ATPase activity by 25% (n=11-12; p<0.05) - Decreased Ca^{2+}ATPase activity (independent of Mg-ATPase activity) by –27% (n=11-12; p<0.05)
			Ambient	Calcium 40uM +ATP 50um +Ionomycin (800mM)	Yes	CO+FO vs. CO - Decreased $Ca^{2+}Mg^{2+}$ATPase activity by 27% (n=11-12; p<0.05) - Decreased $Ca^{2+}Mg^{2+}$ ATPase activity (independent of Mg^{2+}ATPase activity) by 27% (n=11-12; p<0.05) - NC in Mg-ATPase activity (independent of Ca^{2+}ATPase activity) (n=11-12; p>0.05)

Evidence Table 2. Whole-Animal Isolated Organ and Cell Studies

Part 2

Author, yr	Outcome Category	Cell or Tissue	Expt Condition	Agent	Change in Membrane Composition	Results (n=cells)
			Ambient	40uM calcium+ 1mM ATP +Ionomycin (800mM)	Yes	CO+FO vs. CO - Decreased $Ca^{2+}Mg^{2+}$ATPase activity by 23% (n=11-12; p<0.05) - Decreased Ca^{2+}ATPase activity (independent of Mg^{2+}ATPase activity) by 23% (n=11-12; p<0.05) - Decreased Mg^{2+}ATPase activity (independent of Ca^{2+}ATPase activity (%=ND); (n=11-12; p<0.05) - Decreased calcium accumulation by isolated SR by 27% (n=11-12; p<0.05) - NC in ionomycin stimulation (n=11-12; p>0.05) - NC in acylphosphate (EP) (n=4; p>0.05) - Decreased turnover (Ca-ATPase/total EP) by 24% (n=11-12; p<0.05) - Decreased turnover (Ca-ATPase/CaEP) by 17% (n=11-12; p<0.05) - NC in coupling (ATP/Ca uptake) (n=11-12; p<0.05)

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Evidence Table 3. Isolated Organ And Cell Culture Studies

Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
Bayer, 1979	Germany, U	Cat Adult heart in situ	ALA-Na 2mg/kg/min Free IV	5 min	BEP	Ambient	INDO	ALA vs. Ctrl - No change in intra-atrial conduction time - No change in atrioventricular conduction time - No change in functional refractory period of the atrium - No change in functional refractory period of atrio-ventricular conducting system
Bogdanov, 1998	Russia/USA U	Rat Adult Ventricular	DHA 5µM Free	3-12 mins	ICU	Ambient	None	DHA vs. Ctrl - Decreased I_{to} by 40% (n=ND; p=ND) which were not observed when 4-AP (5µM/ND) was present in the bath medium - Decreased I_{to} amplitude by 60% (n=ND; p=ND) - Increased I_{to} delay (n=ND; p=ND) - Decreased time constant of I_{to} inactivation (t) evoked by a voltage step from –70 to +60MV by 33% within 3mins (n=4; p<0.02). - Effects were reversible by BSA
			DHA 5µM Free	3-12 mins	ICU	Ambient	INDO (10µM) Added with FA	DHA+INDO vs. Ctrl+INDO - Presence of INDO did not modify effects on I_{to} indicating that effects of DHA were not related to its cyclooxygenase products (n=ND; p>0.05)
			DHA 50µM Free	3-12 mins	ICU	Ambient	None	DHA vs. Ctrl - Decreased I_{SUS} by 32% (n=ND; p=ND) - No change in I_{Kl} at voltages between -120 to -80mV (n=5; p>0.05)
			EPA 5-10µM Free	3-12 mins	ICU	Ambient	None	EPA vs. Ctrl - No change in of I_{SUS} (n=4; p>0.05)
			EPA 20µM Free	3-12 mins	ICU	Ambient	None	EPA vs. Ctrl - Decreased I_{SUS} by 16% (n=4; p<0.05)
			EPA 50µM Free	3-12 mins	ICU	Ambient	None	EPA vs. Ctrl - Decreased I_{to} by 73% (n=4; p<0.05) - Decreased I_{SUS} by 56% (n=4; p<0.05) - No change in I_{Kl} at voltages between -120 to -80mV (n=5; p>0.05)
			EPA 5-10um Free	10-15 mins	BEP	Ambient	None	EPA vs. Ctrl - Increased AP (% in fig) (n=ND; p=ND) - No change in APA (n=ND; p>0.05)
			EPA 20uM Free	10-15 mins	BEP	Ambient	None	EPA vs. Ctrl - Increased APD (% in fig) (n=ND; p=ND) - Decreased APA (% in fig) (n=ND; p<0.05) - Decreased V_{max} (% in fig) (n=ND; p=ND)
			DHA 10-50µM Free	10-15 mins	BEP	Ambient	None	DHA vs. Ctrl - Similar effects as EPA on APD, APA and V_{max} (data not shown)

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Evidence Table 3. Isolated Organ And Cell Culture Studies

Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
Courtois, 1992	France G	Rat Neonatal Ventricular	SM3-Na-BSA (ALA+EPA) 28.3+29.9 % of total FA's Bound	24 hour s	CP	Ambient	None	SM3 vs. Ctrl - No change in contraction rate (n=5; p>0.05) - No change in CD_{80} (n=5; p>0.05) - No change $+C_{max}$ (n=5; p>0.05) - No change in $-C_{max}$ (n=5; p>0.05) SM3 vs. SM6 - No change in contraction rate (n=5; p>0.05) - No change in CD_{80} (n=5; p>0.05) - Increased $+C_{max}$ (n=5; p<0.01) - No change in $-C_{max}$ (n=5; p>0.05)
			SM3-Na-BSA (ALA+EPA) 28.3+29.9 % of total FA's Bound	24 hour s	CP	Ambient	ISO (10^{-7} M) Added after FA	SM3+ISO vs. Ctrl+ISO - Decreased contraction rate by10% (n=5; p<0.05) - No change in CD_{80} (n=5; p>0.05) - No change in $+C_{max}$ (n=5; p>0.5) - No change in $-C_{max}$ (n=5; p>0.05) SM3+ISO vs. SM6+ISO - No change in contraction rate (n=5; *p>0.05*) - No change in CD_{80} (n=5; *p>0.05*) - No change in $+C_{max}$ (n=5; *p>0.05*) - No change in $-C_{max}$ (n=5; *p>0.05*) SM3+Iso vs. SM3 - Increased contraction rate by 18% (n=5; p<0.01) - Decreased CD_{80} by –12% (n=5; p<0.01) - No change $+C_{max}$ (n=5; p>0.05) - Decreased $-C_{max}$ by 13% (n=5; p<0.01)
de Jonge, 1996	Netherlands G	Rat Neonatal Ventricular	EPA 214µM Bound	4-5 days	CP	Ambient	None	EPA vs. Ctrl - Decreased irregularity of spontaneous contractions (n=4; p<0.05)
Durot, 1997	France G	Rat Neonatal Ventricular	SM3 media containing 25µM EPA-Al + 25µM DHA-Al Bound	4 days	BEP	Ambient	None	SM3 vs. SM6 - Increased V_{max} by 16% (n=9; p<0.05) - No change in MDP (n=9; p>0.05) - No change in OS (n=9; p>0.05) - No change in AP (n=9; p>0.05) - No change in APA (n=9; p>0.05) - No change in APD_{40} (n=9; p>0.05) - No change in APD_{80} (n=9; p>0.05)

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Evidence Table 3. Isolated Organ And Cell Culture Studies

Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
			SM3 media containing 25µM EPA-Al + 25µM DHA-Al Bound	4 days	BEP	Hypoxia (N_2)	None	SM3 vs. SM6 - Decreased APA (% in fig)(n=5; p<0.05) - Decreased APD_{40} (% in fig) (n=5; p<0.01) - Decreased APD_{80} (% in fig)(n=5; p<0.05) - No change in MDP (n=5; p>0.05) - No change in AP (n=5; p>0.05) - No change in upstroke velocity (n=5; p>0.05) - No change in V_{max} (n=5; p>0.05)
Durot, 1997	France G	Rat Neonatal Ventricular	SM3 media containing 25µM EPA-Al + 25µM DHA-Al Bound	4 days	BEP	Reoxy (O_2 for 1.5 hrs)	None	SM3 vs. SM6 - No change in APA (n=5; p>0.05) - No change in APD_{40} (n=5; p>0.05) - No change in APD_{80} (n=5; p<0.05) - Recovery of MDP was significantly increased i.e. improvement (n=5, p<0.01) - No change in V_{max} (n=5; p>0.05)
			SM3 media containing 25µM EPA-Al + 25µM DHA-Al Bound	4 days	CP	Ambient	None	SM3 vs. SM6 - No change in tC_{20} (n=6, p>0.05) - No change in CD_{20} (n=6, p>0.05) - No change in CD_{80} (n=6, p>0.05) - No change in $+C_{max}$, (n=6, p>0.05) - No change in $-C_{max}$ (n=6, p>0.05)
			SM3 media containing 25µM EPA-Al + 25µM DHA-Al Bound	4 days	CP	Hypoxia (N_2)	None	SM3 vs. SM6 - No change in tC_{20} (n=6, p>0.05) - No change in CD_{20} (n=6, p>0.05) - No change in CD_{80} (n=6, p>0.05) - No change in $+C_{max}$ (n=6, p>0.05) - No change in $-C_{max}$ (n=6, p>0.05)
			SM3 media containing 25µM EPA-Al + 25µM DHA-Al Bound	4 days	CP	Reoxy (O_2 for 1.5 hrs)	None	SM3 vs. SM6 - No change in tC_{20} (n=6, p>0.05) - No change in CD_{20} (n=6, p>0.05) - No change in CD_{80} (n=6, p>0.05) - No change in $+C_{max}$ (n=6, p>0.05) - No change in $-C_{max}$ (n=6, p>0.05)
Ferrier, 2002	Canada G	Guinea Pig Adult Ventricular	DHAm.e. 10µM Free	20 mins	ICU	Ambient	None	DHAm.e. vs. Ctrl - Inhibition in the magnitude of the peak $I_{ca.L}$ by 85% (n=18-24; p<0.001)

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Evidence Table 3. Isolated Organ And Cell Culture Studies

Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
			DHAm.e. 10µM Free	20 mins	CP	Ambient	None	DHAm.e. vs. Ctrl - Decreased amplitude of CICR induced contractions by 93% (n=18-24; p<0.001) - No change in VSRM induced contractions (n=18-24; p>0.05)
Fournier, 1995	France G/ NP	Rat Neonatal Ventricular	EPA 100µM Bound DHA 100µM Bound	4 days	CP	Ambient	None	EPA vs. DHA - No change in tC_{20} (n=11; p>0.05) - No change in CD_{20} (n=11; p>0.05) - No change in CD_{80} (n=11; p>0.05) - No change in $+C_{max}$ (n=11; p>0.05) - No change in $-C_{max}$ (n=11; p>0.05)
Fournier, 1995	France G/ NP	Rat Neonatal Ventricular	EPA 100µM Bound DHA 100µM Bound	4 days	BEP	Ambient	None	EPA vs. DHA - Increased APA due to a higher plateau phase (%in fig) (n=11; p<0.05) - Increased OS (% in fig) (n=11; p<0.05) - No change in MDP (n=11;p>0.05) - No change in ADP_{40} (n=11;p>0.05) - No change in ADP_{80} (n=11;p>0.05) - No change in AP (n=11;p>0.05) - No change in V_{max} (n=11;p>0.05)
Goel, 2002	Canada G/NP	Pig Adult Ventricular SL vesicles	EPA 10µM Free	90+/- 30s	IPIM	Ambient	None	EPA vs. Ctrl - No change in H^+dependent Na^+ uptake (Na^+/H^+exchange) (n=3-5; p>0.05)
			EPA 25µM Free	90+/- 30s	IPIM	Ambient	None	EPA vs. Ctrl - No change in H^+dependent Na^+ uptake (Na^+/H^+exchange) (n=3-5; p>0.05)
			EPA 50µM Free	90+/- 30s	IPIM	Ambient	None	EPA vs. Ctrl - Decreased H^+dependent Na^+ uptake by 24% (Na^+/H^+exchange) (n=3-5; p<0.05) which occurred at all reaction times (2-60 secs) and at all extravesicular pH values except pH 6 - No change in passive Na^+ efflux (n=6; p>0.05)
			EPA 100µM Free	90+/- 30s	IPIM	Ambient	None	EPA vs. Ctrl - Decreased H^+dependent Na^+ uptake (Na^+/H^+exchange) (% in fig) (n=3-5; p<0.05) which occurred at all reaction times (2-60 secs) and at all extravesicular pH values except pH 6
			DHA 10µM Free	90+/- 30s	IPIM	Ambient	None	DHA vs. Ctrl - No change in H^+dependent Na^+ uptake (Na^+/H^+exchange) (n=3-5; p>0.05)
			DHA 25µM Free	90+/- 30s	IPIM	Ambient	None	DHA vs. Ctrl - Decreased H^+dependent Na^+ uptake (Na^+/H^+exchange) (% in fig) (n=3-5; p<0.05)

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Evidence Table 3. Isolated Organ And Cell Culture Studies

Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
			DHA 50µM Free	90+/- 30s	IPIM	Ambient	None	DHA vs. Ctrl - Decreased H^+dependent Na^+ uptake (Na^+/H^+exchange) by 34% (n=3-5; p<0.05) - No change in passive Na^+ efflux (n=6; p>0.05)
			DHA 100µM Free	90+/- 30s	IPIM	Ambient	None	DHA vs. Ctrl - Decreased H^+dependent Na^+ uptake (Na^+/H^+exchange) (% in fig) (n=3-5; p<0.05)
			ALA 50µM Free	90+/- 30s	IPIM	Ambient	None	ALA vs. Ctrl - No change in H^+dependent Na^+ uptake (Na^+/H^+exchange) (n=3-5; p>0.05)
			DHA 50µM Free	90+/- 30s	IPIM	Ambient	Na^+ (0.05, 25 or 10mM)	DHA vs. Ctrl - Decreasd H^+ dependent Na^+ uptake (Na^+/H^+exchange) as a function of Na^+ by 30-40% (n=3-4; p<0.05)
Grynberg, 1988	France G	Rats Neonatal Ventricular	SM3 media containing 57% ALA+7%LA +0.2% AA as Na-Al	24 hours	BEP	Ambient	None	SM3 vs. SM6 - No change in AP (n=11, p>0.05) - No change in APA (n=11, p>0.05) - No change in APD_{40} (n=11, p>0.05) - No change in APD_{80} (n=11, p>0.05) - No change in MDP (n=11, p>0.05) - No change in OS (n=11, p>0.05) - No change in V_{max} (n=11, p>0.05)
			SM3 media containing 57% ALA+7%LA +0.2% AA as Na-Al	24 hours	BEP	Hypoxia (N_2)	None	SM3 vs. SM6 - No change in AP (n=11; p>0.05) - Decreased APA (n=11, p<0.01). - No change in APD_{40} (n=11, p>0.05) - No change in APD_{80} (n=11, p>0.05) - No change in MDP (n=11, p>0.05) - Decreased OS (n=11, p<0.05) - No change in V_{max} (n=11, p>0.05)
			SM3 media containing 57% ALA+7%LA +0.2% AA as Na-Al	24 hours	BEP	Reoxy (O_2)	None	SM3 vs. SM6 - No change in AP (n=11, p>0.05) - Increased APA (n=11, p<0.01) - No change in APD_{40} (n=11, p>0.05) - No change in APD_{80} (n=11, p>0.05) - No change in MDP (n=11, p>0.05) - Increased OS (n=11, p<0.05) - No change in V_{max} (n=11, p>0.05)

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Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
			SM3 media containing 57% ALA+7%LA +0.2% AA as Na-Al	24 hours	CP	Ambient	None	SM3 vs. SM6 - No change in tC_{20} (n=11, p>0.05) - No change in CD_{80} (n=11, p>0.05)
			SM3 media containing 57% ALA+7%LA +0.2% AA as Na-Al	24 hours	CP	Hypoxia (N_2)	None	SM3 vs. SM6 - No change in tC_{20} (n=11, p>0.05) - No change in CD_{80} (n=11, p>0.05)
			SM3 media containing 57% ALA+7%LA +0.2% AA as Na-Al	24 hours	CP	Reoxy (O_2)	None	SM3 vs. SM6 - No change in tC_{20} (n=11, p>0.05) - No change in CD_{80} (n=11, p>0.05)
Grynberg, 1995	France G/NP	Rat Neonatal Ventricular	EPA-Na-BSA 100µM DHA-Na-BSA 100µM Bound	4 days	CP	Ambient	None	EPA vs. DHA - No change in spontaneous beating frequency (n=12; p>0.05) - No change in CD_{20} (n=12; p>0.05) - No change in CD_{80} (n=12; p>0.05) - No change in $+C_{max}$ (n=12; p>0.05) - No change in $-C_{max}$ (n=12; p>0.05)
			EPA-Na-BSA 100µM DHA-Na-BSA 100µM Bound	4 days	CP	Ambient	ISO (10^{-7} M) Added after FA	EPA+ISO vs. DHA+ISO - Decreased spontaneous beating frequency by 40% (n=6; p<0.05) - No change in normalized CD_{80} (n=6; p>0.05) EPA+ISO vs. EPA - Increased spontaneous beating frequency by 30% (n=6; p<0.05) DHA+ISO vs. DHA - Increased spontaneous beating frequency by 50% (n=6; p<0.05)
			EPA-Na-BSA 100µM DHA-Na-BSA 100µM Bound	4 days	CP	Ambient	Phe (3×10^{-6} M) Added after FA	EPA+Phe vs. DHA+Phe - No change in spontaneous beating rate (n=6; p>0.05) - No change in CD_{80} (n=6; p>0.05)

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Evidence Table 3. Isolated Organ And Cell Culture Studies

Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
			EPA-Na-BSA 100µM DHA-Na-BSA 100µM Bound	4 days	CP	Ambient	dBcAMP (10^{-73}M) Added after FA	EPA+dBcAMP vs. DHA+dBcAMP - Decreased spontaneous beating rate (% in fig) (n=6; p<0.05) EPA+dBcAMP vs. EPA - Increased spontaneous beating rate by 40% (n=6; p=ND) DHA+dBcAMP vs. DHA - Increased spontaneous beating rate by 60% (n=6; p=ND)
Grynberg, 1996	France U	Rat Neonatal Ventricular	EPA-Albumin 0.1mM Bound DHA-Albumin 0.1mM Bound	4 days	CP	Ambient	None	- EPA vs. DHA - No change in spontaneous rate (n=10; p>0.05) - No change in CD_{20} (n=10; p>0.05) - No change in CD_{80} (n=10; p>0.05) - No change in $+C_{max}$ (n=10; p>0.05) - No change in $-C_{max}$ (n=10; p>0.05)
			EPA-Albumin 0.1mM Bound	4 days	CP	Ambient	Phe ($3x10^{-6}$M)	EPA+Phe vs. DHPA+Phe - No change in contraction rate (n=10; p>0.05)
Grynberg, 1996	France U	Rat Neonatal Ventricular	EPA-Albumin 0.1mM Bound DHA-Albumin 0.1mM Bound	4 days	CP	Ambient	ISO (10^{-6}M)	EPA+ISO vs. DHA+ISO - Decreased contraction rate (% in fig) (n=10; p<0.01)
			EPA-Albumin 0.1mM Bound DHA-Albumin 0.1mM Bound	4 days	CP	Ambient	dBcAMP (10^{-3}M)	EPA+dBcAMP vs. DHA+dBcAMP - Decreased contraction rate (% in fig) (n=10; p<0.01)

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Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
			EPA-Albumin 0.1mM Bound DHA-Albumin 0.1mM Bound	4 days	BEP	Ambient	None	EPA vs..DHA - Increased APA by 3% (n=10; p<0.05) - Increased OS by 13% (n=10; p0<0.05) - No change in MDP (n=10; p>0.05) - No change in APD_{80} (n=10; p>0.05) - No change in V_{max} (n=10; p>0.05)
Hallaq, 1990	USA/Germany G	Rat Neonatal Cardiac	EPA 5µM Bound	3-5 days	CP	Ambient	None	EPA vs. Ctrl - No change in amplitude of contraction (n=6; *p>0.05*) - No change in beats/min (n=6; *p>0.05*)
			EPA 5µM Bound	3-5 days	CP	Ambient	OUA (0.1mM) Added after FA	EPA+OUA vs. Ctrl+OUA - Increased amplitude of contraction by 156% (n=6; *p<0.001*) - Decreased beats/min by 67% (n=6; *p<0.001*) EPA vs. EPA+OUA - Increased amplitude of contraction by 33% (n=6; *p<0.001*) - Decreased beats/min by 31% (n=6; *p<0.001*)
			EPA 5µM Bound	3-5 days	IPIM	Ambient	None	EPA vs. Ctrl - No change in cytosolic free Ca^{2+} (n=8; p>0.05)
			EPA 5µM Bound	3-5 days	IPIM	Ambient	OUA (1µM) Added after FA	EPA+OUA vs. Ctrl+OUA - No change in time averaged cytosolic free Ca^{2+} induced by OUA (n=3; *p>0.05*)
			EPA 5µM Bound	3-5 days	IPIM	Ambient	OUA (0.1mM) Added after FA	EPA+OUA vs. Ctrl+OUA - Decreased time averaged cytosolic free Ca^{2+} induced by OUA by 75% (n=5; *p<0.001*)
Hallaq, 1990	USA/Germany G	Rat Neonatal Cardiac	EPA 5µM Bound	3-5 days	IPIM	Ambient	OUA (0.1mM) Added after FA	EPA+OUA vs. Ctrl+OU A - No change in OUA sensitive Na, K-ATPase (pump activity) measured as the rate of influx of ^{86}Rb into myocytes (n=10; p>0.05) - No change in OUA sensitive Na, K-ATPase (pump activity) measured using NADH-coupled enzyme assay to determine rate of ATP hydrolysis by Na, K-ATPase (n=3; p>0.05)
			EPA 5µM Bound	3-5 days	IPIM	Ambient	BUME (10µM) Added after FA	EPA+BUME vs. Ctrl+BUME - No change in BUME sensitive Na, K-ATPase (pump activity) measured as the rate of influx of ^{86}Rb into myocytes which also indicates that the facilitated cotransport pathway for Na^+, K^+ and $2Cl^-$ is not affected by EPA (n=11; p>0.05)
			EPA 5µM Bound	3-5 days	IPIM	Ambient	OUA+BUME (0.1mM+10 µM) Added after FA	EPA+OUA+BUME vs. Ctrl+OUA+BUME - No change in total Na, K-ATPase (pump activity) measured as the rate of influx of ^{86}Rb into myocytes (n=11; p>0.05)

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Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
Hallaq, 1992	USA G	Rat Neonatal Ventricular	DHA 5µM Free	1-2mins	CP	Ambient	None	DHA vs. Ctrl - No change in contractility (n=6; p>0.05)
			DHA 5µM Free	1-2mins	CP	Ambient	OUA (0.1mM) Added before or after FA	DHA+OUA vs. Ctrl+OUA - Prevented or Terminated arrhythmia's (n=10; p<0.05)
			DHA 5µM Free	1-2mins	CP	Ambient	NIT (0.5nM) Added with FA	DHA+NIT vs. NIT - Prevented the inhibitory effect of NIT on contractility (n=6; p<0.05)
			DHA 5µM Free	1-2mins	CP	Ambient	BAY (0.1µM) Added after FA	DHA+BAY vs. Ctrl+BAY - Prevented the inhibitory effects of BAY on contractility (n=4; p<0.05)
			DHA 5µM Free	1-2mins	CP	Ambient	VER (10µM) or DIL (1µM) Added with FA	DHA+VER or DIL vs. Ctrl+VER or DIL - Did not prevent the inhibitory effects of VER or DIL on contractility (n=3-4; p=ND)
			EPA 5µM Free	1-2mins	CP	Ambient	OUA (0.1mM) Added after FA	EPA+OUA vs. Ctrl+OUA - Prevented arrhythmia (n=ND; p<0.05)
			EPA 5µM Bound	4 days	ICH	Ambient	NIT (0.03^{-10} nM) Added after FA	EPA vs. Ctrl - Noncompetitive inhibition of the specific binding of NIT by reducing the maximal binding of [^{3}H] NIT - Decreased K_d value of high affinity binding site by 97% (n=5-10; *p<0.05*) - Decreased the number of high affinity binding sites (B_{MAX}) by –90% (n=5-10; *p<0.01*) - Decreased K_d value of low affinity binding site by 74% (n=5-10; *p<0.01*) - Decreased the number of low affinity binding sites (B_{MAX}) by 60% (n=5-10; *p<0.05*)
Hallaq, 1992	USA G	Rat Neonatal Ventricular	DHA 5µM Bound	4 days	ICH	Ambient	NIT (0.03^{-10} nM) Added after FA	DHA vs. Ctrl - K_d value of high affinity binding site was non detectable due to suppression by DHA (n=5-10; *p<0.001*) - Number of high affinity binding sites (B_{MAX}) was non detectable due to suppression by DHA (n=5-10; *p<0.001*) - Decreased K_d value of low affinity binding site by 78% (n=5-10; *p<0.01*) - Decreased the number of low affinity binding sites (B_{MAX}) by 64% (n=5-10; *p<0.05*)
			DHA 5uM Bound	4 days	IPIM	Ambient	OUA (0.1mM) $^{45}Ca2^{+}$	DHA+OUA vs. OUA - Decreased $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) by 29% (n=4-11; p<0.025)
			DHA 5µM Bound	4 days	IPIM	Ambient	NIT (0.5nM) Added after FA	DHA+NIT vs. Ctrl+NIT - Increased $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) by 28% (n=5-14; p=ND) - DHA+NIT vs. DHA - No change in $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) (n=5-14; *p>0.05*)

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Evidence Table 3. Isolated Organ And Cell Culture Studies

Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
			DHA 5µM Bound	4 days	IPIM	Ambient	BAY (0.1µM) Added after FA	DHA+BAY vs. Ctrl+BAY - Decreased $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) by 32% (n=5-14; p=ND) DHA+BAY vs. DHA - No change in $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) (n=5-14; *p>0.05*)
			DHA 5µM Bound	4 days	IPIM	Ambient	OUA+ NIT (0.1mM+0.5 nM) Added after FA	DHA+OUA+NIT vs. Ctrl+OUA+NIT - Increased $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) by 13% (n=5-14; p=ND) DHA+OUA+NIT vs. DHA - No change in $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) (n=5-14; *p>0.05*)
			DHA 5µM Bound	4 days	IPIM	Ambient	BAY+ NIT (0.1µM+ 0.5 nM) Added after FA	DHA+BAY+NIT vs. Ctrl+BAY+NIT - Increased $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) by 55% (n=5-14; p=ND) DHA+Bay+NIT vs. DHA - No change in $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) (n=5-14; *p>0.05*)
			EPA 5µM Bound	4 days	IPIM	Ambient	NIT (0.5nM) Added after FA	EPA+NIT vs. Ctrl+NIT - Increased $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) by 34% (n=5-14; p=ND) EPA+NIT vs. EPA - No change in $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) (n=5-14; *p>0.05*)
			EPA 5µM Bound	4 days	IPIM	Ambient	BAY (0.1µM) Added after FA	EPA+BAY vs. Ctrl+BAY - Decreased $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) by 30% (n=5-14; p=ND) EPA+BAY vs. EPA - No change in $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) (n=5-14; *p>0.05*)
			EPA 5µM Bound	4 days	IPIM	Ambient	OUA+ NIT (0.1mM+0.5 nM) Added after FA	EPA+OUA+NIT vs. Ctrl+OUA+NIT - Increased $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) by 20% (n=5-14; p=ND) EPA+OUA+NIT vs. EPA - No change in $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) (n=5-14; *p>0.05*)
			EPA 5µM Bound	4 days	IPIM	Ambient	BAY+ NIT (0.1µM+0.5 nM) Added after FA	EPA+BAY+NIT vs. Ctrl+BAY+NIT - Increased $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) by 39% (n=5-14; p=ND) EPA+BAY+NIT vs. EPA - No change in $^{45}Ca^{2+}$ uptake (Ca^{2+} influx) (n=5-14; *p>0.05*)
Honore, 1994	France G/NP	Mouse Neonatal Ventricular	DHA 30µM Free	ND	ICH	Ambient	None	DHA vs. Ctrl - Blocked delayed rectifier K^+ channel (Kv1.5) activity (% in fig) (n=5-11; p<0.05)
			ALA ND ND	ND	ICH	Ambient	None	ALA vs. Ctrl - No change in delayed rectifier K^+ channel (Kv1.5) activity (n=ND; p>0.05)
			DHA 30µM Free	ND	ICU	Ambient	None	DHA vs. Ctrl - Intracellular DHA included in the pipette medium did not alter the Kv1.5 current (n=9; p>0.05) - Addition of DHA to the external medium inhibited the Kv1.5 current within 20 seconds indicating that binding occurs on an external site (n=9; p<0.05)

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Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
			DHA 30µM Free	ND	ICU	Ambient	None	DHA vs. Ctrl - No change in inward rectifier K^+ current (n=4; p>0.05) - Decreased ultra rapid K^+ current (I_{KUR}) measured at +30mV by 52% (n=4; p<0.05)
Jahangiri, 2000	Australia U	Rat Adult Atrial	EPA 10µM Free	7 mins	CP	Ambient	ISO (10µM) Added with FA	EPA+ISO vs. Ctrl+ISO - Decreased the number of asynchronously contracting atrial myocytes by 76% (n=107/7hearts; p<0.01)
			DHA 10µM Free	7 mins	CPr	Ambient	ISO (10µM) Added with FA	DHA+ISO vs. Ctrl+ISO - Decreased the number of asynchronously contracting atrial myocytes by 69% (n=101/5 hearts; p<0.05)
			DHA m.e 10µM Free	7 mins	CP	Ambient	ISO (10µM) Added with FA	DHA m.e+ISO vs. Ctrl+ISO - No change in the number of asynchronously contracting atrial myocytes (n=71/4 hearts; p>0.05)
Juan, 1987	Austria U	Guinea Pigs Adult Isolated heart	EPA-Na $6x10^{-8}$mol/min Free	30mins	CP	Ambient	Antigen-ovalbumin (1mg/0.1ml) Added before FA	EPA+Antigen vs. Ctrl+Antigen - No change in duration of arrhythmia (n=8; p>0.05)
			EPA-Na $15x10^{-8}$mol/min Free	30mins	CP	Ambient	Antigen-ovalbumin (1mg/0.1ml) Antioxidant-esculetin ($1x10^{-7}$mol) Added before FA	EPA+antigen vs. Ctrl+Antigen - Decreased duration of arrhythmia by 56% (n=8; p<0.05) EPA+Antioxidant+Antigen vs. Ctrl+Antigen - Decreased duration of arrhythmia by 52% (n=5; p<0.05)
Kang, 1994	USA G	Rat Neonatal Cardiac	EPA 5-10µM Free	3 mins	CP	Ambient	None	EPA vs. Ctrl - Decreased contraction rate by 50 to 80% within 2 mins (n=46; p<0.05) and effects were reversed by BSA - No change in amplitude of contraction (n=ND; p>0.05)
			DHA 5-10µM Free	3 mins	CP	Ambient	None	DHA vs. Ctrl - Decreased contraction rate by 50 to 80% within 2 mins (n=32; p<0.05) and effects were reversed by BSA - No change in amplitude of contraction (n=ND; p>0.05)
Kang, 1994	USA G	Rat Neonatal Cardiac	EPA 5-10µM Free	3 mins	CP	Ambient	INDO (10-20µM/) BW755c (20 µM) BHT (0.005%/ Vitamin E (0.5uNIT/ml) and ETYA (ND) Added with FA	EPA+agents vs. Ctrl+agents - No change in EPA induced reductions in beating rate (n=ND; p>0.05)

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Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
			EPA 5-10µM Free	3 mins	CP	Ambient	Ca^{2+} (7-10µM) Added before or after FA	EPA+Ca^{2+} vs. Ctrl+Ca^{2+} - Prevented or Terminated arrhythmia (n=ND; p<0.05) and the effects were reversible by BSA
			DHA 5-10µM Free	3 mins	CP	Ambient	Ca^{2+} (7-10µM) Added before or after FA	DHA+Ca^{2+} vs. Ctrl+Ca^{2+} - Prevented or Terminated arrhythmia (n=ND; p<0.05) and the effects were reversible by BSA
			EPA 5-10µM Free	3 mins	CP	Ambient	OUA (0.1mM) Added before FA	EPA+OUA vs. Ctrl+OUA - Terminated contractures/fibrillations (n=ND; p<0.05) and the effects were reversible by BSA
			DHA 5-10µM Free	3 mins	CP	Ambient	OUA (0.1mM) Added before FA	DHA+OUA vs. Ctrl+OUA - Terminated contractures/fibrillation (n=ND; p<0.05) and the effects were reversible by BSA
			ALA 5-10µM Free	3 mins	CP	Ambient	None	ALA vs. Ctrl - Decreased beating rate by 40% (mean of the range (n=5; p<0. 05) and the effects were reversible by BSA
			EPA e.e 5-10µM Free	3 mins	CP	Ambient	None	EPAe.e vs. Ctrl - No change in beating rate (n=3; p>0.05)
Kang, 1995a	USA G	Rats Neonatal Ventricular	EPA 10µM Free	2-5 mins	BEP	Ambient	None	EPA vs. Ctrl - Hyperpolarizing RMP by 5±1 mV (n=8, p<0.05). The effect was reversible by BSA (2mg/ml). - Depolarizing APT by 9±3 mV (n=8, p<0.05). The effect was reversible by BSA (2mg/ml). - Decreased APD_{75} by 21% (n=8, p<0.01) - No change in APA (n=8, p>0.05) - No change in V_{max} (n=8, p>0.05) - Decreased action-potential frequency by 50% after 3 minutes EPA addition (n=8, p<0.05) - Increased the stimulation strengths required to initiate action potentials by 49% (n=ND, p<0.01) Effect was reversible by BSA
Kang, 1995b	USA G/NP	Rat Neonatal Cardiac	EPA 5-10µM Free	5mins	CP	Ambient	ISO (3uM) Added before or after FA	EPA+ISO vs. ISO - Prevented or Terminated arrhythmia within 2-3 mins (n=8; p<0.05) - Decreased contraction rate (%=ND) (n=5; p=ND) - Effects were reversible by BSA
Kang, 1995b	USA G/NP	Rat Neonatal Cardiac	EPA 5-10µM Free	5 mins	CP	Ambient	ISO (3uM)+ INDO (20 uM/)+ BW (ND) Added before FA	EPA+ISO+INDO+BW vs. Ctrl+ISO+INDO+BW - Prevented arrhythmia (n=3; p<0.05)

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Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
			DHA 5-10µM Free	5 mins	CP	Ambient	ISO (3uM)+ INDO (20 uM/)+ BW (ND) Added before FA	DHA+ISO+INDO+BW vs. Ctrl+ISO+INDO+BW - Prevented arrhythmia (n=3; p<0.05)
			EPA 5-10µM Free	5 mins	CP	Ambient	cAMP (250uM) Added after FA	EPA+cAMP vs. Ctrl+cAMP - Terminated arrhythmias w/in 3-5min (n=5; p<0.05)
			EPA 8µM Free	5 mins	CP	Ambient	Cholera toxin (2ug/ml) (Gs protein activator)	EPA+Cholera toxin vs. Cholera toxin - Decreased beating rate (%=ND) (n=4; p=ND) - Effects were reversed by BSA
Kang , 1996	USA G	Rat Neonatal Cardiac	EPA 10-15µM Free	3-7 mins	CP	Ambient	LPC (5-10 µM) Added before or 3-5 mins after FA	EPA+LPC vs. Ctrl+LPC - Prevented tachycardia and slowed beating rate with 2-3 mins and also terminated arrhythmia (n=5; p<0.05) - Effects were reversible by BSA
			DHA 10-15µM Free	3-7 mins	CP	Ambient	LPC (5-10 µM) Added before FA	DHA+LPC vs. Ctrl+LPC - Prevented tachycardia and slowed beating rate with 2-3 mins (n=5; p<0.05) - Effects were reversible by BSA
			ALA 10-15µM Free	3-7 mins	CP	Ambient	LPC (5-10 µM) Added before FA	ALA+LPC vs. Ctrl+LPC - Prevented tachycardia and slowed beating rate with 2-3 mins (n=5; p<0.05) - Effects were reversible by BSA
			EPA 10-15µM Free	3-7 mins	CP	Ambient	PTC (2-10 µM) Added before or after FA	EPA+PTC vs. Ctrl+LPC - Prevented or Terminated occurrence of arrhythmia (n=5;p<0.05) - Effects were reversible by BSA
			DHA 10-15µM Free	3-7 mins	CP	Ambient	PTC (2-10 µM) Added before or after FA	DHA+PTC vs. Ctrl+LPC - Prevented or Terminated occurrence of arrhythmia (n=5;p<0.05)
			ALA 10-15µM Free	3-7 mins	CP	Ambient	PTC (2-10 µM) Added before or after FA	ALA+PTC vs. Ctrl+LPC - Prevented or Terminated occurrence of arrhythmia (n=5;p<0.05)
			EPA 10-15µM Free	7mins	CP	Ambient	Ca^{2+}ionophore (5µM) Added before FA	EPA+ Ca^{2+} vs. Ctrl+ Ca^{2+} - Prevented or Terminated occurrence of arrhythmia (n=5;p<0.05)
			EPA 15µM Free	3-5mins	CP	Ambient	Electrical pacing (15V)	EPA vs. Ctrl - Decreased electrical automaticity/ excitability of the cardiac myocyte by 50% (n=7; p<0.01)
Kang , 1996	USA G	Rat Neonatal Cardiac	EPA 10-15µM Free	7mins	IPIM	Ambient	None	EPA vs. Ctrl - No change in systolic and diastolic (cytosolic) Ca^{2+} (n=6; p>0.05)

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Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
			EPA 10-15µM Free	7mins	IPIM	Arr	LPC (5-10 µM) Added before FA	EPA+LPC vs. Ctrl+LPC - Terminated intermittent fluctuation of Ca^{2+} (n=6; p<0.05)
Kang, 1997	USA G	Rats Neonatal Cardiac	EPA 20µM Bound	3-4 days	ICH	Ambient	None	EPA vs. Ctrl - No change in the number of Na+ channels per 10^6 cell, measured by the binding of [^{3}H] BTXB (n=4, p>0.05)
			EPA 20µM Bound	3-4 days	ICH	Ambient	MEX (20 uM)	EPA+MEX vs. Ctrl+ MEX - Decreased the number of Na+ channels per 10^6 cell by 40% to 50% (n=4, p<0.05) - Decreased the MEX induced increase in cardiac Na^+ channel expression
Leifert, 1999	Australia U	Rat Adult Ventricular	DHA 25µM Free	4 mins	ICU	Ambient	None	DHA vs. Ctrl - Decreased I_{Na} peak current amplitude by 42% (n=7; p=ND)
			DHA 25µM Free	4 mins	ICU	Ambient	None	DHA vs. Ctrl - Shifted the voltage dependence of I_{Na} activation to more positive potentials as indicated by a decrease in G_{mas} by 35% and a shift of V' to a more positive potentialby 21% (n=5; p<0.01) - Shifted the voltage dependence of of I_{Na} inactivation to more negative potentials as indicated by a decrease in I_{max} by 36% and a shift of V' to more hyperpolarized potentials by 30% (n=5; p<0.01)
			EPA 25µM Free	4 mins	ICU	Ambient	None	EPA vs. Ctrl - Shifted the voltage dependence of I_{Na} activation to more positive potentials as indicated by a decrease in G_{mas} by-30% and a shift of V' to a more positive potential by 26% (n=10; p<0.001) - Shifted the voltage dependence of of I_{Na} inactivation to more negative potentials as indicated by a decrease in I_{max} by 35% and a shift of V' to more hyperpolarized potentials by 25% (n=10; p<0.01)
			ALA 25µM Free	4 mins	ICU	Ambient	None	ALA vs. Ctrl - Shifted the voltage dependence of I_{Na} activation to more positive potentials as indicated by a decrease in G_{mas} by 18% and a shift of V' to a more positive potential by 25% (n=6; p<0.001) - Shifted the voltage dependence of of I_{Na} inactivation to more negative potentials as indicated by a decrease in I_{max} by 25% and a shift of V' to more hyperpolarized potentials by 30% (n=6; p<0.01)
Leifert, 2000b	Australia U	Rat Adult Ventricular	DHA 10µM Free	ND	CP	Ambient	ISO (10um) Added 5 mins after FA	DHA+ISO vs. Docasanoic Acid+ISO - Decreased spontaneous contractions by 85% (n=5; p<0.01)
			DHA 10µM Free	ND	CP	Ambient	LPC (10um) Added 5 mins after FA	DHA+LPC vs. Stearic Acid+LPC - Decrease in spontaneous contractions by 77% (n=4; p<0.01)

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Leifert, 2000b	Australia U	Rat Adult Ventricular	DHA 10μM Free	ND	CP	Ambient	Electrical Stimulation (1Hz at 25 V)	DHA vs. Stearic Acid - Decrease in asynchronous contractions by 61% (n=4; p<0.05)
Li, 1997	USA G	Rat Neonatal Cardiac	EPA 10μM Free	ND	CP	Ambient	Eicosanoids PGD2+PGE2+PGF2 +U46619 (3μm-0.5μM)	EPA vs. Ctrl - Terminated the arrhythmias and contractures within 2-3 minutes (n=ND, p<0.05), followed by a slow beating rate.
Macleod, 1998	New Zealand G/NP	Rat Adult Ventricular	EPA 1-7.5uM Free	5 mins	CP	Ambient	None	EPA vs. Ctrl - Increased (prolonged) twitch size (%=ND) (n=6-8; p=ND)
			DHA 1-7.5uM Free	5 mins	CP	Ambient	None	DHA vs. Ctrl - Increased (prolonged) twitch size (%=ND) (n=6-8; p=ND)
			EPA >10M Free	5 mins	CP	Ambient	None	EPA vs. Ctrl - Decreased twitch size (%=ND) (n=6-8; p=ND)
			DHA >10M Free	5 mins	CP	Ambient	None	DHA vs. Ctrl - Decreased twitch size (%=ND) (n=6-8; p=ND)
			EPA 1-7.5uM Free	5 mins	BEP	Ambient	None	EPA vs. Ctrl - Dose dependant increase (lengthening of early plateau potential) in ADP_{80} (%=ND) (n=11-14; p=ND)
			DHA 1-7.5uM Free	5 mins	BEP	Ambient	None	DHA vs. Ctrl - Dose dependant increase (lengthening of early plateau potential) in ADP_{80} (%=ND) (n=11-14; p=ND)
			EPA >10M Free	5 mins	BEP	Ambient	None	EPA vs. Ctrl - Dose dependant decrease in ADP_{80} (%=ND) (n=11-14; p=ND)
			DHA >10M Free	5 mins	BEP	Ambient	None	DHA vs. Ctrl - Dose dependant decrease in ADP_{80} (%=ND) (n=11-14; p=ND)
			EPA 5,10 or 20uM Free	5 mins	ICU	Ambient	None	EPA vs. Ctrl - Dose dependant decrease of the peak amplitude of the I_{Na} (%=ND) (n=6-8; p=ND)
			DHA 5,10 or 20uM Free	5 mins	ICU	Ambient	None	DHA vs. Ctrl - Dose dependant decrease of the peak amplitude of the I_{Na} (%=ND) (n=6-8; p=ND)

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			EPA 5,7.5 or 10uM Free	5 mins	ICU	Ambient	None	EPA vs. Ctrl - Dose dependant decrease of the peak $I_{Ca.L}$ (%=ND) (n=5-8; p=ND)
Macleod, 1998	New Zealand G/NP	Rat Adult Ventricular	DHA 5,7.5 or 10uM Free	5 mins	ICU	Ambient	None	DHA vs. Ctrl - Dose dependant decrease of the peak $I_{Ca.L}$ (%=ND) (n=5-8; p=ND)
			EPA 0.1-10uM Free	5 mins	ICU	Ambient	None	EPA vs. Ctrl - Dose dependant decrease of I_{to} (%=ND) (n=5-8; p=ND)
			DHA 0.1-10uM Free	5 mins	ICU	Ambient	None	DHA vs. Ctrl - Dose dependant decrease of I_{to} (%=ND) (n=5-8; p=ND)
			EPA 2um Free	5 mins	ICU	Ambient	None	EPA vs. Ctrl - Decreased I_K and I_{K1} by 30-40%(n=ND; p=ND)
			EPA 5um Free	5 mins	ICU	Ambient	None	EPA vs. Ctrl - Decreased I_K and I_{K1} by 50-60% (n=ND; p=ND)
		Guinea Pig Adult Ventricular	EPA 5-20µM Free	5 mins	CP	Ambient	None	EPA vs. Ctrl - Dose dependant decrease in twitch size (%=ND) (n=6-8; p=ND)
			DHA 5-20µM Free	5 mins	CP	Ambient	None	DHA vs. Ctrl - Dose dependant decrease in twitch size (%=ND) (n=6-8; p=ND)
			EPA 1-20µM Free	5 mins	BEP	Ambient	None	EPA vs. Ctrl - Dose dependant reduction in ADP_{80} (%=ND) (n=12-16; p=ND)
			DHA 1-20µM Free	5 mins	BEP	Ambient	None	DHA vs. Ctrl - Dose dependant reduction in ADP_{80} (%=ND) (n=12-16; p=ND)
			EPA 5,10 or 20µM Free	5 mins	ICU	Ambient	None	EPA vs. Ctrl - Dose dependant decrease of the peak amplitude of I_{Na} (%=ND) (n=8-10; p=ND)
			DHA 5,10 or 20µM Free	5 mins	ICU	Ambient	None	DHA vs. Ctrl - Dose dependant decrease of the peak amplitude of I_{Na} (%=ND) (n=8-10; p=ND)

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			EPA 5,7.5 or 10µM Free	5 mins	ICU	Ambient	None	EPA vs. Ctrl - Dose dependant decrease of the peak $I_{Ca.L}$ (%=ND) (n=6-10; p=ND)
Macleod, 1998	New Zealand G/NP	Guinea Pig Adult Ventricular	DHA 5,7.5 or 10µM Free	5 mins	ICU	Ambient	None	DHA vs. Ctrl - Dose dependant decrease of the peak $I_{Ca.L}$ (%=ND) (n=6-10; p=ND)
			EPA 2um Free	5 mins	ICU	Ambient	None	EPA vs. Ctrl - Decreased I_K and I_{Kl} by 10% (n=5-8; p=ND)
			EPA 5um Free	5 mins	ICU	Ambient	None	EPA vs. Ctrl - Decreased I_K and I_{Kl} by 30-40% (n=5-8; p=ND)
Negretti, 2000	Venezuela G/NP	Rat Adult Ventricular	EPA 10µM Free	3 mins	CP	Ambient	None	EPA vs. Ctrl - Increased resting cell length by 2% (n=6, p<0.001). - Decreased the spontaneous contraction frequency (n=47 out of 57; p<0.001) - Effects were reversible by BSA
			EPA 10µM Free	3 mins	IPIM	Ambient	None	EPA vs. Ctrl - Decreased the frequency of spontaneous waves of calcium release (n=47 out of 57; p<0.001) - Decreased the amplitude of the wave by 16% (n=41, p<0.001) - Effects were reversible by BSA
			EPA 10µM Free	3 mins	IPIM	Ambient	Ca^{2+} (10uM)	EPA vs. Ctrl - Decreased the basal level of [Ca2+] by 6% (n=46, p<0.005). The effect was reversible by BSA.
			EPA 5µM Free	3 mins	IPIM	Ambient	Caffeine (10mM)	EPA vs. Ctrl - Increased the SR calcium content indicated by an increase in the caffeine induced Na^{+}-Ca^{2+} exchange current by 41% (n=4, p<0.05).
			DHA 5µM Free	3 mins	IPIM	Ambient	Caffeine (10mM)	DHA vs. Ctrl - Increased the SR calcium content indicated by an increase in the caffeine induced Na^{+}-Ca^{2+} exchange current by 41% (n=4, p<0.05).
			EPA 10µM Free	3 mins	ICU	Ambient	None	EPA vs. Ctrl - Decreased the amplitude of $I_{Ca.L}$ (n=5; p<0.05)
			DHA 10µM Free	3 mins	ICU	Ambient	None	DHA vs. Ctrl - Decreased the amplitude of $I_{Ca.L}$ (n=5; p<0.05)

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Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
O'Neill, 2002	UK/Venezuela G/NP	Rat ND Ventricular	EPA 10uM Free	ND	ICU	Ambient	None	EPA vs. Ctrl - Decreased frequency of transient inward currents that accompany spontaneous waves of CICR by 33% (n=6; p<0.05) - Increased amplitude of currents activated by each wave by 29% (n-6; p<0.05)
			EPA 10uM Free	ND	IPIM	Ambient	None	EPA vs. Ctrl - Decreased resting cytosolic Ca^{2+} due to decrease Ca^{2+} influx across surface membrane and not due to increased activation of efflux pathways(n=6; p<0.05)
O'Neill, 2002	UK/Venezuela G/NP	Rat ND Ventricular	EPA 10uM Free	ND	IPIM	Ambient	None	EPA vs. Ctrl - Decreased wave frequency activated by Ca2+ efflux (% in fig) (n=6; p<0.01) - Increased efflux of Ca2+ activated by individual waves by 12% (n=6; p<0.05) - Decreased wave generated efflux per unit time by 19% (n=6; p<0.01) - Decreased total efflux (% in fig) (n=6; p<0.01)
			EPA 10uM Free	ND	IPIM	Ambient	Caffeine (10mM/ND)	EPA+Caffeine vs. Ctrl+Caffeine - No change in surface membrane Ca2+ efflux pathway (n=12; p>0.1)
Pepe, 1994	USA G	Rat Young Adult Cardiac	DHA 5µM Free	4 mins	CP	Ambient	None	DHA vs. Ctrl - No change in DL (n=6, p>0.05) - No change in TA_{t50} (n=6, p>0.05) - No change in VS/DL (n=6; p>0.05)
			DHA 5µM Free	4 mins	CP	Ambient	NIT (10nM)	DHA+NIT vs. Ctrl+NIT - Blocked NIT effect on TA (n=6, p<0.05) - Blocked NIT effect on VS/DL (n=6; p<0.05) - Effects were reversible by BSA
			DHA 5µM Free	4 mins	CP	Ambient	BAY (10nM)	DHA+BAY vs. Ctrl+BAY - Blocked NIT effect on TA (n=6, p<0.05) - Blocked NIT effect on VS/DL (n=6; p<0.05) - Effects were reversible by BSA
			DHA 5µM Free	4 mins	CP	Ambient	ISO (0.1-1uM)	DHA+ISO vs. Ctrl+ISO - No change in TA (n=6, p>0.05) - No change in DL (n=6; p>0.05) - Effects were reversible by BSA
			DHA 5µM Free	4 mins	ICU	Ambient	None	DHA vs. Ctrl - No change in peak $I_{Ca.L}$ amplitude (n=6; p>0.05) -
			DHA 5µM Free	4 mins	ICU	Ambient	NIT (10nM)	DHA+NIT vs. Ctrl+NIT - Increased peak $I_{Ca.L}$ amplitude by 50% (n=6; p<0.05) - Effects were reversible by BSA
			DHA 5µM Free	4 mins	ICU	Ambient	BAY (10nM)	DHA+BAY vs. Ctrl+BAY - Blocked the BAY induced increase in peak $I_{Ca.L}$ amplitude (n=6; p<0.05) - Effects were reversible by BSA

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Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
			DHA 5µM Free	4 mins	ICU	Ambient	ISO (0.1-1uM)	DHA+ISO vs. Ctrl+ISO - No change in peak $I_{Ca.L}$ amplitude (n=6; p>0.05) -
			DHA 5µM Free	4 mins	IPIM	Ambient	None	DHA vs. Ctrl - No change in $IFTA_{dias}$ and IFR $_{t50}$ indicating no change in cytosolic Ca^{2+} and Cai^{2+} transient amplitude (n=6; p>0.05)
			DHA 5µM Free	4 mins	IPIM	Ambient	NIT (10nM)	DHA+NIT vs. Ctrl+NIT - Inhibited NIT blockage of the L-type calcium channel influx (n=6; p<0.05) - Blocked NIT effect on $IFTA_{dias}$ (n=6; p<0.05) - Effects were reversible by BSA
Pepe, 1994	USA G	Rat Young Adult Cardiac	DHA 5µM Free	4 mins	IPIM	Ambient	BAY (10nM)	DHA+BAY vs. Ctrl+BAY - Inhibited BAY induced potentiation of L-type calcium channel influx (n=6; p<0.05) - Blocked BAY effect on $IFTA_{dias}$ (n=6; p<0.05) - Effects were reversible by BSA
			DHA 5µM Free	4 mins	IPIM	Ambient	ISO (0.1-1uM)	DHA+ISO vs. Ctrl+ISO - No change in ISO induced increase in cytosolic calcium content (n=6; p>0.05)
Phiipson, 1985	USA G	Dog Adult Ventricular SL vesicles	ALA 30µM Free	1.5 sec	IPIM	Ambient	Ca^{2+}(10uM) Added before FA	ALA vs. Ctrl - Increased Na^{+}-Ca^{2+} exchange measured as Na^{+} dependent Ca^{2+} uptake by 112% (n=9, p<0.05) - Preincubation with ALA to ensure complete incorporation resulted in the max imal stimulation of Na+ dependent Ca2+ influx being about 40% less than when the vesicles were only briefly exposed to ALA.
			ALA 20µM Free	2 mins	IPIM	Ambient	Preloaded Ca^{2+} (47.9 nMl)	ALA vs. Ctrl - Increased passive Ca2+ efflux by 147% (n=3, p<0.05)
Phiipson, 1987	USA G	Dog Adult Ventricular SL vesicles	ALA 60µM Free	1.5 sec	IPIM	Ambient	Ca^{2+}(10uM)	ALA vs. Ctrl - Increased Na^{+}-Ca^{2+} exchange measured as Na^{+} dependent Ca^{2+} uptake by 87% (n=3, p<0.05)
			ALA 30µM Free	2 minutes	IPIM	Ambient	Preloaded Ca^{2+} (52.3 nM)	ALA vs. Ctrl - Increased passive Ca2+ efflux by 170% (n=4, p<0.05)
Ponsard, 1999	France NP	Rat Neonatal Ventricular	EPA+DHA-Albumin 4.6+6.5% Bound	4 days	CP	Ambient	None	EPA+DHA vs. Ctrl - No change in CR (n=13; *p>0.05*) - No change in CD_{20} (n=13; *p>0.05*) - No change in CD_{80} (n=13; *p>0.05*) - No change in $+C_{max}$ (n=13; *p>0.05*) - No change in $-C_{max}$ (n=13; *p>0.05*)

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			EPA+DHA-Albumin 4.6+6.5% Bound	4 days	CP	Ambient	ISO (10^{-7}M)	EPA+DHA+ISO vs. N-6+ISO - Increased CR by 66% (n=7; p<0.05) - No change in CD_{20} (n=7; p>0.05) - No change in CD_{80} (n=7; p>0.05) - No change in $+C_{max}$ (n=7; p>0.05) - No change in $-C_{max}$ (n=7; p>0.05)
			EPA+DHA-Albumin 4.6+6.5% Bound	4 days	CP	Ambient	PHE (10^{-6}M)	EPA+DHA+PHE vs. N-6+PHE - Increased CR by 115% (n=6; p<0.05) - No change in CD_{20} (n=6; p>0.05) - No change in CD_{80} (n=6; p>0.05) - No change in $+C_{max}$ (n=6; p>0.05) - No change in $-C_{max}$ (n=6; p>0.05)
Ponsard, 1999	France NP	Rat Neonatal Ventricular	EPA+DHA-Albumin 4.6+6.5% Bound	4 days	CP	Normoxia-Posthypoxic Reoxy	ISO (10^{-7}M)	EPA+DHA+ISO in Hypoxia vs. EPA+DHA+ISO in Normoxia - Increased CR (% in fig) (n=6; p<0.001) - No change in CD_{20} (n=6; p>0.05) - No change in CD_{80} (n=6; p>0.05) - Increased $+C_{max}$ (%=ND) (n=6; p<0.05) - No change in $-C_{max}$ (n=6; p>0.05)
			EPA+DHA-Albumin 4.6+6.5% Bound	4 days	CP	Normoxia-Posthypoxic Reoxy	PHE (10^{-6}M)	EPA+DHA+PHE in Hypoxia vs. EPA+DHA+PHE in Normoxia - No change (n=6; p>0.05) - No change in CD_{20} (n=6; p>0.05) - No change in CD_{80} (n=6; p>0.05) - No change in $+C_{max}$ (n=6; p>0.05) - No change in $-C_{ax}$ (n=6; p>0.05)
Reithman, 1996	Germany U	Rat Neonatal Cardiac	DHA 60µM Bound	3 days	BEP	Ambient	None	DHA vs. Ctrl - Increased amplitude by 20% (n=28-29; p<0.05) - No change in APR (n=14-19; p>0.05)
			DHA 60µM Bound	3 days	BEP	Ambient	NA + TIM (100µmol/L+10 µmol/L)	DHA+NA+TIM vs. Ctrl+NA+TIM - Decreased APR by 28% (n=14-19; p<0.05)
			DHA 60µM Bound	3 days	CP	Ambient	NA + TIM (100µmol/L+10 µmol/L)	DHA+NA+TIM vs. Ctrl+NA+TIM - Decreased arrhythmias by 84% (n=15-28; p<0.01)
			DHA 60µM Bound	3 days	BEP	Ambient	Isoprenaline (10 µmol/L)	DHA+Isoprenaline vs. Ctrl+Isoprenaline - Decreased APR by 26% (n=10-11; p<0.05)
			DHA 60µM Bound	3 days	BEP	Ambient	OUA (10 µmol/L)	DHA+OUA vs. Ctrl+OUA - Decreased APR by 16% (n=4 ; p<.05)

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Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
Rinaldi, 2002	Italy NP	Rat Adult Ventricular	DHA 10µM Free	20 minutes (acute)	IPIM	Ambient	None	DHA vs. Ctrl - - No change in basal cytosolic Ca^{2+} levels (n=9; p>0.5)
			DHA 10µM Free	3 days (chronic)	IPIM	Ambient	None	DHA vs. Ctrl - - No change in cytosolic Ca^{2+} levels (n=9; p>0.5)
			DHA 10µM Free	20 minutes (acute)	IPIM	Ambient	ET-1 (100nM)	DHA+ET-1 vs. Ctrl - Increased ET-1 induced cytosolic Ca^{2+} levels by 128% (n=9; p<0.01)
			DHA 10µM Free	3 days (chronic)	IPIM	Ambient	ET-1 (100nM)	DHA+ET-1 vs. Ctrl - Increased ET-1 induced cytosolic Ca^{2+} by 148% (n=9; p<0.01)
			DHA 10µM Free	20 minutes (acute)	IPIM	Ambient	KCl (50mM) Added after FA	DHA+KCl vs. Ctrl - Decreased Ca2+ by 71% (n=9; p<0.01)
Rinaldi, 2002	Italy NP	Rat Adult Ventricular	DHA 10µM Free	3 days (chronic)	IPIM	Ambient	KCl (50mM) Added after FA	DHA+KCl vs. Ctrl+KCl - Decreased Ca^{2+} by 48% (n=9; p<0.01)
			DHA 10µM Free	20 minutes (acute) 3 days (chronic)	IPIM	Ambient	KCl (50mM) Added after FA	DHA+ET-1 (chronic) vs. DHA+ET-1 (acute) - Decreased Ca^{2+} by 17% (n=9; p<0.01)
			DHA 10µM Free	20 minutes (acute)	IPIM	Anoxia	97%N_2 and 3% CO_2	DHA+Anoxic Soln vs. Ctrl - Decreased Ca^{2+} by 58% (n=9; p<0.01)
			DHA 10µM Free	3 days (chronic)	IPIM	Anoxia	97%N_2 and 3% CO_2	DHA+Anoxic Soln vs. Ctrl - Decreased Ca^{2+} by 83% (n=9; p<0.01)
			DHA 10µM Free	20 minutes (acute) 3 days (chronic)	IPIM	Anoxia	97%N_2 and 3% CO_2	DHA+Anoxic Soln (chronic) vs. DHA+Anoxic Soln (acute) - Decreased Ca^{2+} by 59% (n=9; p<0.01)
			DHA 10µM Free	20 minutes (acute)	IPIM	Anoxia	97%N_2 and 3% CO_2+ KCl (50mM)	DHA+Anoxic Soln+KCl vs. Ctrl - Decreased Ca^{2+} (%=ND) (n=9 ;p<0.01)
			DHA 10µM Free	3 days (chronic)	IPIM	Anoxia	97%N_2 and 3% CO_2+ KCl (50mM)	DHA+Anoxic Soln+KCl vs. Ctrl - Decreased Ca^{2+} (%=ND) (n=9; p<0.01)

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			DHA 10µM Free	20 minutes (acute) 3 days (chronic)	IPIM	Anoxia	97%N_2 and 3% CO_2+ KCl (50mM)	DHA+Anoxic Soln+KCl (chronic) vs. DHA+Anoxic Soln+KCl (acute) - Decreased Ca^{2+} by 70% (n=9; p<0.01)
			DHA 10µM Free	20 minutes (acute)	IPIM	Anoxia	97%N_2 and 3% CO_2+ ET-1 (100nM)	DHA+Anoxic Soln+ET-1 vs. Ctrl - Decreased Ca^{2+} (%=ND) (n=9; p<0.01)
			DHA 10µM Free	3 days (chronic)	IPIM	Anoxia	97%N_2 and 3% CO_2+ ET-1 (100nM)	DHA+Anoxic Soln+ET-1 vs. Ctrl - Decreased Ca^{2+} (%=ND) (n=9; p<0.01)
			DHA 10µM Free	20 minutes (acute) 3 days (chronic)	IPIM	Anoxia	97%N_2 and 3% CO_2+ ET-1 (100nM)	DHA+Anoxic Soln+ET-1 (bound) vs. DHA+Anoxic Soln+ET-1 (free) - Decreased Ca^{2+} by 70% (n=9; p<0.01)
			ALA-Na 2mg/kg/min Free as IV	5 mins	CP	Ambient	None	ALA-Na vs. Ctrl - No change in intra-atrial conduction time (AC) (n=7; p>0.05) - No change in atrio-ventricular conductance time (AVC) (n=7; p>0.05) - No change in functional refractory period of the atrium (ARP) (n=7; p>0.05) - No change in functional refractory period of atrio-ventricular conducting system (AVRP) (n=7; p>0.05)
Rodrigo, 1999	New Zealand G/NP	Rat Adult Ventricular	EPA 5µM Free	10 mins	CP	Ambient	None	EPA vs. Ctrl - Decreased twitch contraction size by 70 (n=8; p<0.001) - Effects were reversible by BSA
			EPA 5µM Free	10 mins	ICU	Ambient	None	EPA vs. Ctrl - Decreased $I_{Ca.L}$ by 72% (n=8; p<0.001) - Effects were reversible by BSA
		Guinea Pig Adult Ventricular	EPA 5µM Free	10 mins	CP	Ambient	None	EPA vs. Ctrl - Initial increase in twitch contraction size (% cell shortening) followed by a decrease in twitch contraction strength by –88% (n=7; p<0.001) - Effects were partially reversible by BSA
		Guinea Pig Adult Ventricular	EPA 5µM Free	10 mins	ICU	Ambient	None	EPA vs. Ctrl - Decreased $I_{Ca.L}$ by 64% (n=11; p<0.001) - Effects were reversible by BSA

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		Rat Adult Skinned/ Saponin Permealized Ventricular	EPA 5uM Free	10mins	CP + IPIM	Ambient	Ca^{2+} (133-267nM) Added before FA	EPA+Ca^{2+} vs. Ctrl+Ca^{2+} - Decreased frequency of spontaneous contractions (%=ND) (n=5; p<0.05) due to an inhibition of SR Ca^{2+} release - No change in degree of relaxation between spontaneous contractions (n=5; p>0.05)
			EPA 10µM Free	10mins	CP+ IPIM	Ambient	Ca^{2+} (133-267nM) Added before FA	EPA+Ca^{2+} vs. Ctrl+Ca^{2+} - Decreased frequency of spontaneous contractions (%=ND) (n=5; p<0.05) due to an inhibition of SR Ca^{2+} release - No change in degree of relaxation between spontaneous contractions (n=5; p>0.05)
		Guinea Pig Adult Skinned/ Saponin Permealized Ventricular	EPA 5µM Free	10 mins	CP	Ambient	Ca^{2+} (133-267nM) Added before FA	EPA+Ca^{2+} vs. Ctrl+Ca^{2+} - Decreased frequency of spontaneous contractions (%=ND) (n=5; p<0.05) due to an inhibition of SR Ca^{2+} release - No change in degree of relaxation between spontaneous contractions (n=5; p>0.05)
Vitelli, 2002	Italy U	Rat Adult Ventricular	DHA 10µM Free	20 mins	IPIM	Ambient	Ca^{2+} free KRB (1.8mM)	DHA vs. Ctrl - No change in basal level of cytosolic Ca2+ (n=ND; p>0.01)
			DHA 10µM Free	20 mins	IPIM	Ambient	$CaCl_2$ KRB (1.8mM)	DHA vs. Ctrl - No change in basal level of cytosolic Ca2+ (n=ND; p>0.01)
Vitelli, 2002	Italy U	Rat Adult Ventricular	DHA 10µM Free	20 mins	IPIM	Ambient	DXR (100uM) Added after FA Ca^{2+} free KRB (1.8mM)	DHA+DXR vs. Ctrl+DXR - Decreased peak level of Ca^{2+} (n=ND; p<0.01) DHA+DXR vs. Ctrl - No change in peak level of Ca^{2+} (n=ND; p>0.05) DHA+DXR vs. DHA - No change in peak level of Ca^{2+} (n=ND; p>0.05)
			DHA 10µM Free	20 mins	IPIM	Ambient	DXR (100uM) Added after FA $CaCl_2$ KRB (1.8mM)	DHA+DXR vx Ctrl+DXR - Decreased peak level of Ca^{2+} (n=9; p<0.01) DHA+DXR vs. Ctrl - No change in peak level of Ca^{2+} (n=9; p>0.05) DHA+DXR vs. DHA - No change in peak level of Ca^{2+} (n=9; p>0.05)
			DHA 10µM Free	20 mins	IPIM	Ambient	Caff (10mM) Added after FA Ca^{2+} free KRB (1.8mM)	DHA+Caff vx Ctrl+Caff - Decreased peak level of Ca^{2+} (n=9; p<0.01) DHA+DXR vs. Ctrl - No change in peak level of Ca^{2+} (n=9; p>0.05)

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			DHA 10µM Free	20 mins	IPIM	Ambient	Caff (10mM) Added after FA $CaCl_2$ KRB (1.8mM)	DHA+Caff vx Ctrl+Caff - Decreased peak level of Ca^{2+} (n=9; p<0.01) DHA+DXR vs. Ctrl - No change in peak level of Ca^{2+} (n=9; p>0.05)
Weylandt, 1996	USA G	Rat Neonatal Cardiac	EPA 15µM DHA 15µM Bound	48 hrs	CP	Ambient	ISO (3-10uM)	EPA+ISO vs. Ctrl+ISO - No change in arrhythmias (n =51-107; p=ND) EPA+ISO vs. DHA+ISO - No change in arrhythmias (n=51-107; p>0.1)
			DHA 15µM Bound	>48 hrs	CP	Ambient	ISO (3-10uM)	DHA+ISO vs. Ctrl+ISO - No change in arrhythmias (n=13 -51; p>0.1)
			EPA 15µM DHA 15µM Bound	48 hrs	CP	Ambient	Ca^{2+} (7mM)	EPA+ Ca^{2+}vs. Ctrl+ Ca^{2+} - No change in arrhythmias (n =14-20; p>0.1) EPA+ Ca^{2+}vs. DHA+ Ca^{2+} - No change in arrhythmias (n=6-14; p>0.1)
			DHA 15µM Bound	48 hrs	CP	Ambient	Ca^{2+} (7mM)	DHA+Ca^{2+} vs. Ctrl+Ca^{2+} - No change in arrhythmias (n=6-20; p>0.1)
			DHA 15µM Free	3-12mins	CP	Ambient	ISO (3-10uM) Added before FA	DHA+ISO vs. Ctrl+ISO - Terminated arrhythmias (n=8; p<0.05)
			EPA 15µM Free	3-12mins	CP	Ambient	ISO (3-10uM) Added before FA	EPA +ISO vs. Ctrl+ISO - Terminated arrhythmias (n=8; p<0.05)
Weylandt, 1996	USA G	Rat Neonatal Cardiac	DHA 15µM Bound EPA 15µM Free	3-12mins 48 hrs	CP	Ambient	ISO (3-10uM) Added before FA	DHA vs. DHA+ISO - Terminated arrhythmias (n=23; p<0.05) EPA vs. EPA+ISO - Terminated arrhythmias (n=23; p<0.05)
			DHA or EPA 15µM Free	3-12mins	CP	Ambient	Ca^{2+} (7mM))	DHA+Ca^{2+} vs. Ctrl+Ca^{2+} - Decreased arrhythmias by -83% (n=12; p<0.05) EPA vs. Ctrl+Ca^{2+} - Decreased arrhythmias by -83% (n=12; p<0.05)

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			DHA or EPA 15µM Bound DHA or EPA 15µM Free	3-12mins 48 hrs	CP	Ambient	Ca^{2+} (7mM)	DHA (free) vs. DHA (bound)+Ca^{2+} - Decrease in arrhythmias by -90% (n=10; p<0.05) EPA (free) vs. EPA (bound)+Ca^{2+} - Decrease in arrhythmias by -90% (n=10; p<0.05)
Xiao, 1995	USA G	Rat Neonatal Ventricular	EPA 5-10µM Free	ND	ICU	Ambient	None	EPA vs. Ctrl - Suppressed voltage activated Na^+ currents within 2mins which was reversible by BSA (n=6; p<0.05) - No change in current-voltage relations or in the activation and inactivation time constants of Na^+ current (n=10; p>0.05)
			EPA 10-40µM Free	ND	ICU	Ambient	None	EPA vs. Ctrl - Suppressed Na^+ current by 68% to 99% with 10um and 40um EPA respectively indicating a dose dependent effect (n=4-10; p<0.05)
			EPA 10µM Free	ND	ICU	Ambient	None	EPA vs..Ctrl - Modified the voltage dependence of the steady state inactivation of I_{Na} (n=7; p<0.001). Inhibition of 83% at –80mV and 29% at –150mV indicating a voltage dependent effect. Application of a train of stimulating pulses at freq.l of 1.0, 0.2, 0.1, or 0.03 Hz had no effect on time required to attain same level of inhibition of I_{Na} independent of concentration (n=5; p>0.05) (time and dose but not use dependent effect)
			EPA 5µM Free	ND	ICU	Ambient	None	EPA vs. Ctrl - Inhibition of I_{Na} by 51% (n=10; p<0.01)
			DHA 5µM Free					DHA vs. Ctrl - Inhibition of I_{Na} by 52% (n=7; p<0.01)
			EPA 10µM Free	ND	ICU	Ambient	None	EPA vs. Ctrl - Inhibition of I_{Na} by 64% (n=21; p<0.001)
Xiao, 1995	USA G	Rat Neonatal Ventricular	DHA 10µM Free	ND	ICU	Ambient	None	DHA vs. Ctrl - Inhibition of I_{Na} by 66% (n=7; p<0.05)
			ALA 10µM Free	ND	ICU	Ambient	None	ALA vs. Ctrl - Inhibition of I_{Na} by 71% (n=5; p<0.05)

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			EPA 1.5µM Free	ND	ICU	Ambient	None	EPA vs. Ctrl - Decreased $I_{Ca,L}$ by 50% (n=11; p<0.05) and effects were partially reversible by BSA - No change in shape of current voltage relationship (n=11; p>0.05) - Negative shift (3.33+/- 0.4 mV) of the $I_{Ca,L}$ inactivation curve (n=11;p<0.05) and effects were reversible by BSA
Xiao, 1997	USA G	Rat Neonatal Ventricular	EPA 0.1-40µM Free	ND	ICU	Ambient	None	EPA vs. Ctrl - Time and dose dependant decrease in $I_{Ca,L}$ within seconds (n=6; p<0.05) - $I_{Ca,L}$ was almost completely inhibited when the concentration of EPA was above 5 uM
			EPA 1µM Free	ND	ICU	Ambient	None	EPA vs. Ctrl - Decreased $I_{Ca,L}$ by 33% when elicited from the holding potential –40 to 0mV than from -80 to 0mV indicating a voltage dependent effect (n=6; p<0.05) - Effect was also time but not frequency or use-dependent (n=4; p>0.05)
			EPA 5µM Free	ND	ICU	Ambient	None	EPA vs. Ctrl - Inhibition of $I_{Ca,L}$ by 83% (n=5; p<0.01) - EPA, DHA and ALA had similar effects on the steady -state inactivation of the calcium cannel (approx 3 to 5 mV shift to negative potentials a the V1/2 point)
			DHA 5µM Free	ND	ICU	Ambient	None	DHA vs. Ctrl - Inhibition of $I_{Ca,L}$ by 62% (n=6; p<0.01)
			ALA 5µM Free	ND	ICU	Ambient	None	ALA vs. Ctrl - Inhibition of $I_{Ca,L}$ by 77% (n=5; p<0.01)
			EPA 1µM Free	ND	ICU	Ambient	None	EPA vs. Ctrl - Suppression of $I_{Ca,L}$ by 57% (n=5; p<0.01)
			EPA 5µM Free	ND	ICU	Ambient	None	EPA vs.. Ctrl - Suppression of $I_{Ca,L}$ by 47% (n=8; p<0.01)
			EPA 1.5µM Free	ND	IPIM	Ambient	None	EPA vs. Ctrl - Decreased the calcium transients induced by $I_{Ca,L}$ (n=ND;p<0.01)
			EPA 15µM Free	ND	ICU	Ambient	None	EPA vs. Ctrl - Decreased the calcium transients induced by $I_{Ca,L}$ (n=ND; p<0.01) - Decreased SR Ca^{2+}release (n=ND; p<0.05) - No change in time constant of decay (tau) or temporal and spatial spread of the calcium sparks (n 33-46; p>0.05) indicating no direct action of EPA on SR Ca^{2+} release or re-uptake
Xiao, 2002	USA G/ NP	Ferret Adult Atrial	DHA 10µM Free	ND	ICU	Ambient	None	DHA vs. Ctrl - Decreased I_{K} by 62% -69% (n=7-12; p<0.05)

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Evidence Table 3. Isolated Organ And Cell Culture Studies

Author, yr	Study Characteristics [Country: Funding:]	Cells [Animal: Age: Type:]	Fatty Acid [N3: Dose: Form:]	Incubation/ Exposure Duration	Outcome Category	Experimental Condition	Agent [Amt]	Results
		Ferret Adult Ventricular	DHA 0.2-50µM Free	ND	ICU	Ambient	None	DHA vs. Ctrl - Dose dependant decrease in I_K (% in fig) (n=6; p<0.05)
			DHA 5µM Free	ND	ICU	Ambient	None	DHA vs. Ctrl - Decreased I_K by 31% (n=12; p<0.05) - No change in I_{K1} (n=6; p>0.05)
			DHA 10µM Free	ND	ICU	Ambient	None	DHA vs. Ctrl - Decreased I_K by 42% regardless of holding potential (n=8; p<0.05) - Decreased I_{to} by 57% (n=7; p<0.001) - No change in I_{K1} (n=5; p>0.05)
			DHA 20µM Free	ND	ICU	Ambient	None	DHA vs. Ctrl - Decreased I_K by 50% (n=6; p<0.01) - No change in I_{K1} (n=2; p>0.05)
			DHA 50µM Free	ND	ICU	Ambient	None	DHA vs. Ctrl - Decreased I_K by 61% (n=11; p<0.001)
			EPA 5µM Free	ND	ICU	Ambient	None	EPA vs. Ctrl - Decreased I_K by 26% (n=6; p<0.05)
			EPA 10µM Free	ND	ICU	Ambient	None	EPA vs. Ctrl - Decreased I_K by 40% (n=8; p<0.001) - Decreased I_{to} by 67% (n=4; p<0.01) - No change in I_{K1} (n=ND; p>0.05)
			ALA 5µM Free	ND	ICU	Ambient	None	ALA vs. Ctrl - Decreased I_K by 22% (n=7; p<0.01)
			ALA 10µM Free	ND	ICU	Ambient	None	ALA vs. Ctrl - Decreased I_K by 46% (n=8; p<0.001) - Decreased I_{to} by 49% (n=4; p<0.05) - No change in I_{K1} (n=ND; p>0.05)
			DHA 10µM Free	ND	ICU	Ambient	Sta (0.1µmol/L) Added before FA	DHA+Sta vs. Ctrl+Sta - Decreased I_K by 65% (n=5; p<0.05)

APPENDIX D. Peer Reviewers

We gratefully acknowledge the following individuals who reviewed the initial draft of this Report and provided us with constructive feedback. Acknowledgments are made with the explicit statement that this does not constitute endorsement of the report.

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