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

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.

Suggested Citation

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References

1. Nair SS, Leitch JW, Falconer J, et al. Prevention of cardiac arrhythmia by dietary (n-3) polyunsaturated fatty acids and their mechanism of action [review]. J Nutr 1997; 127(3):383-93.
2. Hock CE, Beck LD, Bodine RC, et al. Influence of dietary n-3 fatty acids on myocardial ischemia and reperfusion. Am J Physiol 1990; 259(5 Pt 2): H1518-26.
3. Macleod JC, Macknight AD, Rodrigo GC. The electrical and mechanical response of adult guinea pig and rat ventricular myocytes to omega-3 polyunsaturated fatty acids. Eur J Pharmacol 1998; 356(2-3):261-70.
4. Rodrigo GC, Dhanapala S, Macknight AD. Effects of eicosapentaenoic acid on the contraction of intact, and spontaneous contraction of chemically permeabilized mammalian ventricular myocytes. J Mol Cell Cardiol 1999; 31(4):733-43.
5. Bogdanov KY, Spurgeon HA, Vinogradova TM, et al. Modulation of the transient outward current in adult rat ventricular myocytes by polyunsaturated fatty acids. Am J Physiol 1998; 274(2 Pt 2):H571-79.
6. Kang JX, Xiao YF, Leaf A. Free, long-chain, polyunsaturated fatty acids reduce membrane electrical excitability in neonatal rat cardiac myocytes. Proc Natl Acad Sci U S A 1995; 92(9):3997-4001.
7. Reithmann C, Scheininger C, Bulgan T, et al. Exposure to the n-3 polyunsaturated fatty acid docosahexaenoic acid impairs alpha 1-adrenoceptor-mediated contractile responses and inositol phosphate formation in rat cardiomyocytes.
8. O'Neill SC, Perez MR, Hammond KE, et al. Direct and indirect modulation of rat cardiac sarcoplasmic reticulum function by n-3 polyunsaturated fatty acids. J Physiol (Lond) 2002; 538(Pt 1):179-84.
9. Pepe S, Bogdanov K, Hallaq H, et al. Omega-3 polyunsaturated fatty acid modulates dihydropyridine effects on L-type Ca2+ channels, cytosolic Ca2+, and contraction in adult rat cardiac myocytes. Proc Natl Acad Sci U S A 1994; 91(19):8832-6.
10. Rinaldi B, Di Pierro P, Vitelli MR, et al. Effects of docosahexaenoic acid on calcium pathway in adult rat cardiomyocytes. Life Sci 2002; 71(9):993-1004.
11. Leifert WR, McMurchie EJ, Saint DA. Inhibition of cardiac sodium currents in adult rat myocytes by n-3 polyunsaturated fatty acids. J Physiol (Lond) 1999; 520 Pt 3:671-9.
12. Xiao YF, Morgan JP, Leaf A. Effects of polyunsaturated fatty acids on cardiac voltage-activated K(+) currents in adult ferret cardiomyocytes. Sheng Li Hsueh Pao - Acta Physiologica Sinica 2002; 54(4):271-81.
13. Negretti N, Perez MR, Walker D, et al. Inhibition of sarcoplasmic reticulum function by polyunsaturated fatty acids in intact, isolated myocytes from rat ventricular muscle. J Physiol (Lond) 2000; 523(2):367-75.
14. Ferrier GR, Redondo I, Zhu J, et al. Differential effects of docosahexaenoic acid on contractions and L-type Ca2+ current in adult cardiac myocytes. Cardiovasc Res 2002; 54(3):601-10.

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