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.

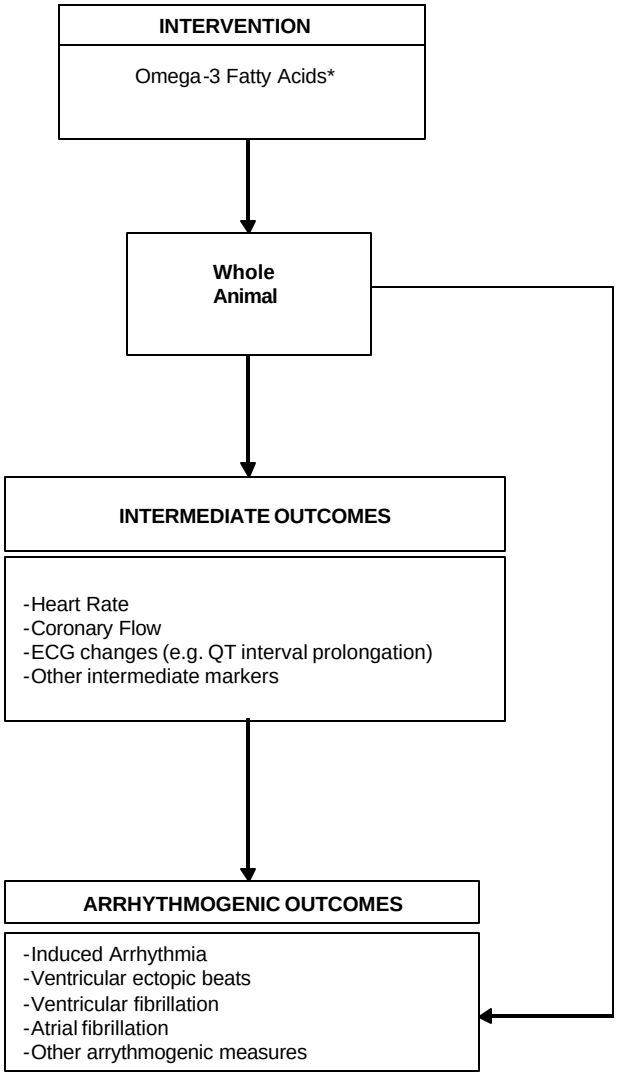
Potential mechanisms suggested by different investigators to explain the antiarrhythmic action of omega-3 fatty acids can be broadly classified into several categories (See list of Acronyms, Abbreviations, and Parameters). These include the effects of omega-3 fatty acids on:

- 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

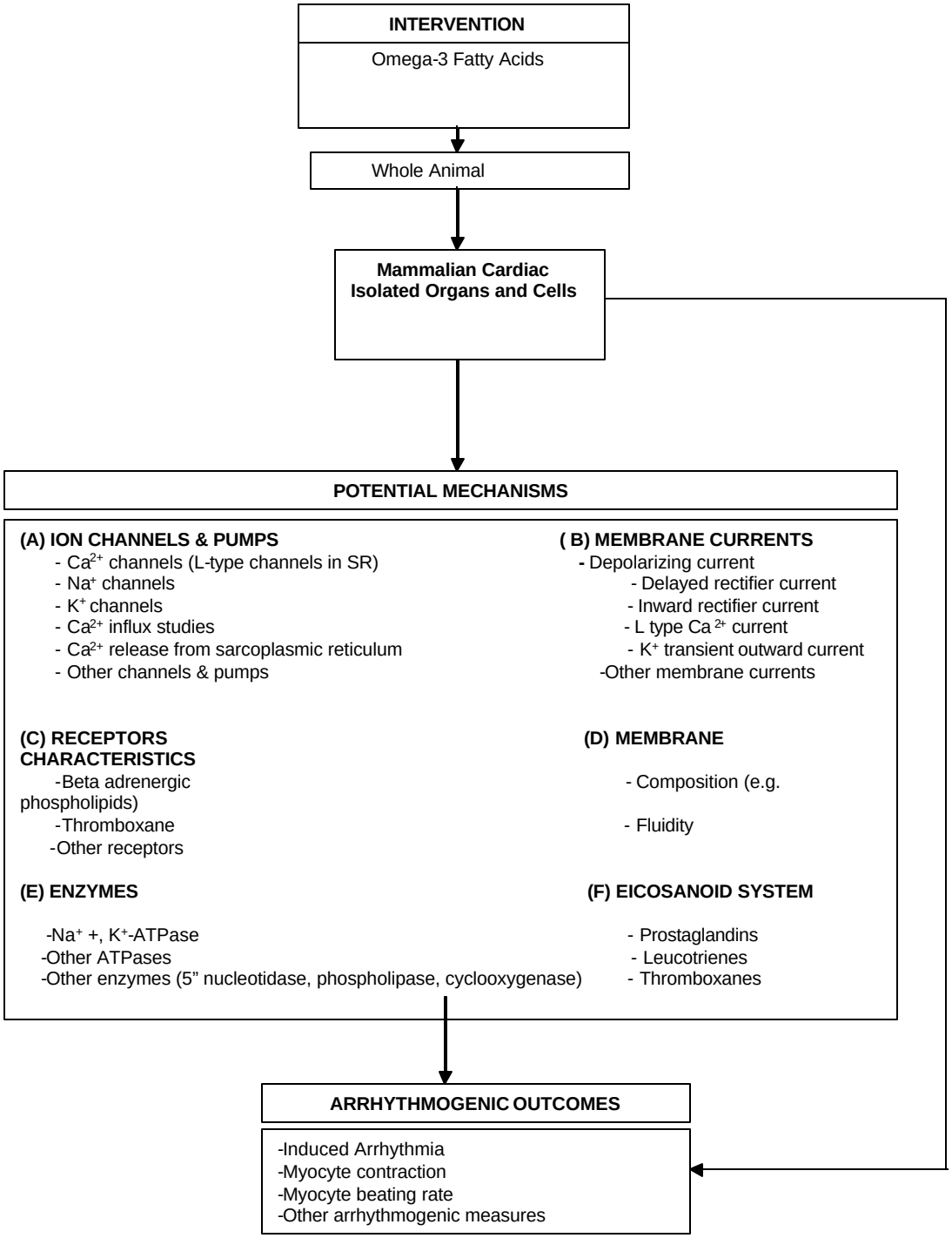
Question: What is the evidence from whole animal studies that omega-3 fatty acids affect arrhythmogenic outcomes (and intermediate outcomes)?



* ALA, EPA, DPA, DHA

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

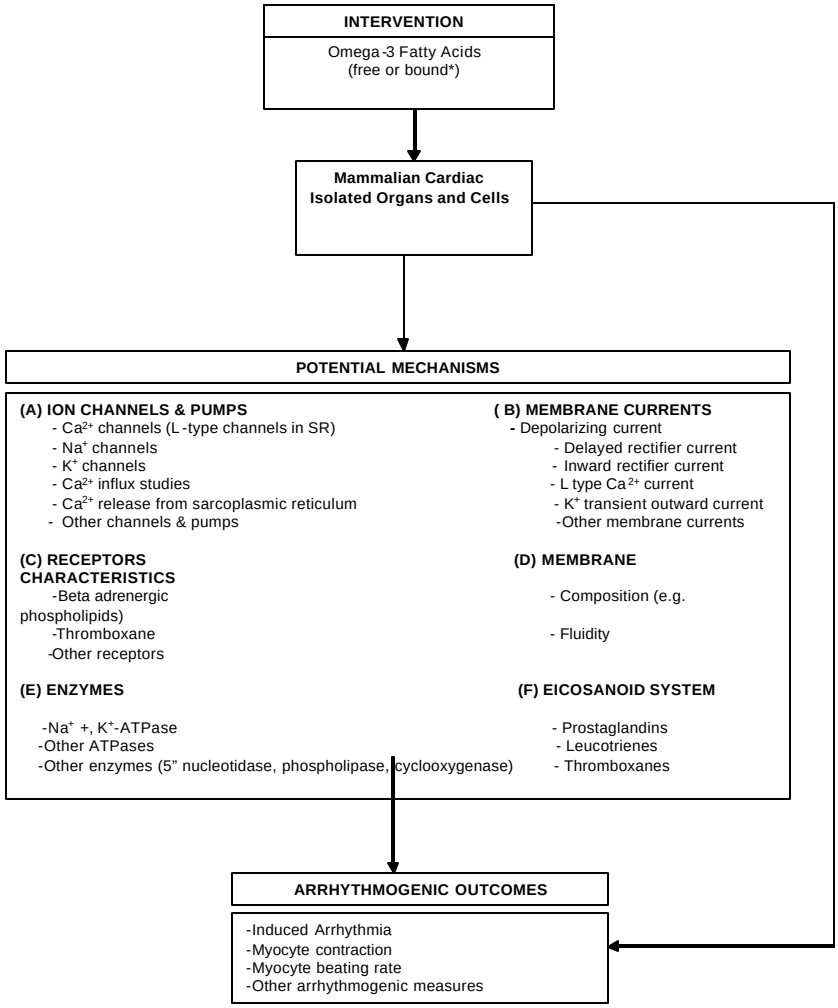
Question: What is the evidence from intact {intact, whole animal} cell culture and tissue studies (including animal and human cardiac tissue). that omega-3 fatty acids directly affect cell organelles such as cardiac ion channels, pumps, or exchange mechanisms involved in electrogenesis?



*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'.