Omega-3 Fatty Acids and their Role in Cardiac Arrhythmogenesis Workshop
Research Challenges and Opportunities

August 29-30, 2005

Embassy Suites Hotel at the Chevy Chase Pavilion
4300 Military Road, NW
Washington, District of Columbia 20015
AGENDA

Omega-3 Fatty Acids and their Role in Cardiac Arrhythmogenesis
Workshop: Research Challenges and Opportunities

Day 1: Monday, August 29, 2005

9:00 a.m.  Call to Order
9:30 a.m.  Welcome and Opening Remarks  Dr. David Lathrop
           Dr. Rebecca Costello
9:35 a.m.  Workshop Goals and Objectives  Dr. Barry London
           (Chair)

Session I - Background: Evidence for Antiarrhythmic
Effects of Omega-3 (n-3) Fatty Acids

9:50 a.m.  Evidence for Antiarrhythmic Effects from Epidemiologic
           Studies  Dr. Christine Albert
10:20 a.m.  Agency for Healthcare Research and Quality
           (AHRQ): Evidence Reports on the Cardiovascular
           Effects of n-3 Fatty Acids  Dr. Ethan Balk
           Ms. Mei Chung
10:50 a.m.  Discussion  All Participants
11:05 a.m.  Break

Session II –NHLBI-supported Trials to Determine the
Antiarrhythmic Effects of n-3 Fatty Acids

11:15 a.m.  The Fatty Acid Antiarrhythmia Trial (FATT) (R01
           HL062154)  Dr. Alexander Leaf
11:55 a.m.  The Antiarrhythmic Effects of n-3 Fatty Acids Study (R01
           HL061682)  Dr. John McAnulty
12:35 p.m.  Discussion  All Participants
12:50 p.m.  Lunch

Session III – Possible Basic Mechanisms of Action

1:50 p.m.  Dietary Source of n-3 Fatty Acids: Metabolic Pathways
           and Sites of Interaction  Dr. Bill Lands
2:20 p.m.  Role of Calcium-Calmodulin Interactions in
           Arrhythmogenesis: Possible Sites of n-3 Fatty Acid
           Modulation  Dr. Mark Anderson
2:50 p.m.  Anti-inflammatory mechanisms of the antiarrhythmic
           effects of n-3 fatty acids  Dr. David Van
           Wagoner
3:20 p.m.  Potassium Channel Targeting to Plasma Membrane
           Lipid Microdomains: Possible n-3 Fatty Acid Effects  Dr. Jeffrey Martens
3:50 p.m. Break
4:10 p.m. Acute n-3 Fatty Acid Effects in Large Animal Models
         Dr. George Billman
4:40 p.m. Possible Sites of n-3 Fatty Acid Actions on
         Electromechanical Activity
         Dr. Wayne Giles
4:40 p.m. Discussion
         Discussion All Participants
5:10 p.m. Summary of Critical Issues Reviewed
         Dr. Barry London
5:45 p.m. Adjourn

Day 2: Tuesday, August 30, 2005

8:30 a.m. Roundtable Discussion and Prioritization of NIH
         Recommendations: Key Issues and Future
         Research Directions, Challenges and
         Opportunities. Develop Specific
         Recommendations to NHLBI/ODS. Select Writing
         Committee
         All Participants
12:30 p.m. Closing Remarks
         Dr. Barry London
1:00 p.m. Adjourn
Purpose:

The major goals for this workshop are to: (1) review the epidemiological evidence and the data from randomized trials on the role of omega-3 fatty acids in susceptibility to arrhythmias and sudden cardiac death; (2) explore the basic mechanisms by which omega-3 fatty acids affect cardiac excitability at the cellular and organ level; (3) identify the gaps and barriers in our basic understanding of the effects of omega-3 fatty acids on cardiac electrical activity at the cellular, tissue, and whole body levels; and (4) provide prioritized recommendations for additional research studies to (a) better understand the basic mechanisms coupling omega-3 fatty acids to cardiac electrical activity and (b) facilitate translation of this knowledge to the treatment and prevention of cardiac arrhythmias.

Public Health Importance:

Cardiac rhythm disturbances are a major public health burden, accounting for well over 250,000 deaths each year due to SCD and the occurrence of approximately 2.2 million cases of atrial fibrillation each year in the United States. The number of people suffering atrial fibrillation is expected to increase to 5.5 million people per year by 2050. Thus it is important to identify promising new therapeutic targets and interventions to treat and prevent cardiac rhythm disturbances.

Workshop Content:

Workshop members will review the present state of knowledge and make recommendations for future approaches to expedite elucidation of the mechanisms of action responsible for the effect of omega-3 fatty acids on cardiac electrical activity and arrhythmogenesis. A summary of the workshop proceedings and recommendations will be prepared for publication in a peer-reviewed, internationally recognized scientific journal.
Session I

Background: Evidence for Antiarrhythmic Effects of Omega-3 (n-3) Fatty Acids
Omega-3 Fatty Acids: Evidence for Antiarrhythmic Effects from Epidemiologic Studies.
Christine Albert, M.D.

2. Where we stand in 2005.

In observational studies, low levels of dietary fish intake (1-2 fish meals per week) along with blood levels of the long-chain n-3 polyunsaturated fatty acids have been associated with reduced risks of sudden cardiac death (SCD) but not non-fatal myocardial infarction (MI). Similar associations have been reported for alpha-linolenic acid (ALA), an intermediate chain n-3 fatty acid found in foods of plant origin, in one study of women but not in men. The specificity of these associations between n-3 fatty acid intake and SCD, as opposed to other types of cardiac events, supports the hypothesis that n-3 fatty acids, particularly the long chain n-3 fatty acids, may influence cardiovascular risk through effects on arrhythmogenesis and fatal ventricular arrhythmias.

In addition to these observational studies, two large randomized trials in MI populations have reported similar findings for the long-chain n-3 fatty acids. The Dart trial found a 29% reduction in mortality without any benefit on non-fatal MI among men randomly assigned to eat at least two portions weekly of fatty fish. More recently the GISSI-Prevenzione trial tested a combination of 850 mg EPA and DHA daily among 11,324 patients with a recent MI. The patients assigned to n-3 PUFA had a significant reduction in the primary endpoint (death, non-fatal MI, and non-fatal stroke) primarily due to statistically significant reduction in SCD (45%) without any benefit on non-fatal MI or stroke. In subsequent sub-group analyses, the benefit on SCD was found to be 4-fold higher in patients with systolic dysfunction (EF ≤ 40%) as compared to those with preserved left ventricular ejection fraction (EF > 50%).

The epidemiologic data examining the association between n-3 fatty acids and atrial arrhythmias are less developed than that for ventricular arrhythmias and SCD, and the data are somewhat conflicting. Negative and positive associations between dietary intake of long chain n-3 fatty acids and risk of atrial fibrillation (AF) have been reported in cohort studies. Only one small randomized trial has been reported among patients after coronary artery bypass grafting, where long chain n-3 fatty acid supplementation significantly reduced post-operative AF. To my knowledge, no study has examined the effect of the shorter chain n-3 fatty acid, ALA, on atrial fibrillation.

Several cross-sectional analyses and small scale clinical trials provide some insights into the mechanisms of action of the long chain n-3 fatty acids in humans. There are data to suggest that higher intakes of n-3 fatty acids via diet or supplementation may influence heart rate, heart rate variability, inflammatory mediators, and directly effect cardiac electrophysiology. However, given the cross sectional nature of many of these studies and the small numbers of patients involved in these mechanistic clinical trials, these data are quite preliminary. The data also conflicts for some of these intermediary markers. In general, significant associations are more likely to be found in studies involving patients with some form of structural heart disease.

Although limited, the above data have prompted the American Heart Association to recommend that all adults eat fish (particularly fatty fish) at least two times per week. In addition, based primarily on the
results of the GISSI-Prevenzione trial, patients with CHD have been advised to consume ~ 1 gram of EPA and DHA (combined) per day, although fish oil supplements have not been directly recommended.

3. Current challenges and the most important issues for future research

The above data, and that from recent randomized trials among ICD patients, suggest that heterogeneity may exist in the antiarrhythmic actions of the n-3 fatty acids. The effects may differ by type of arrhythmia (atrial versus ventricular), underlying cardiac substrate, and/or by sex. Defining the patient populations that benefit from these agents will be an important challenge in the future. Randomized trials in these select patient populations should be a priority. If diets and/or supplements enriched with n-3 fatty were found to have antiarrhythmic properties or to reduce risk of SCD in randomized trials, the public health impact of such a low cost and easily accessible intervention could be significant. Also, since fatty fish is not readily available or palatable to all populations, and concerns have been raised regarding mercury contamination of the fish supply and depletion of ocean fisheries, other sources of n-3 fatty acids should also be investigated in the future.

4. Areas of overlap with other workshop topic areas

There may be overlap with Agency for Health Care Research and Quality (AHRQ): Evidence Reports on the Cardiovascular Effects on n-3 Fatty Acids.

The results of the recent ICD trials will be discussed later on by Dr. Leaf and Dr. McNulty.

5. Translating the topic into understanding effects of n3-fatty acids on arrhythmogenesis

See Section 2 and 3 above.

6. Citations


Omega-3 Fatty Acids
Evidence for Antiarrhythmic Effects from Epidemiologic Studies

Diet and Reinfarction Trial (DART)

Design
- 2033 men under age 70 admitted with AMI.
- Randomized in a factorial design to 3 dietary advice groups: fat, fiber, and fish.
- The Fish advice group were advised to eat at least 2 portions of fatty fish per week (2.5g EPA/wk)

Primary Outcomes:
- Total Mortality
- Total CHD Events (Fatal CHD and non-fatal MI)

Seattle, King County Case-Control Study
Risk of primary cardiac arrest associated with dietary intake of long-chain n-3 PUFA

Relative Risk for Sudden Death According to Dietary Fish Intake

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study of Fish Consumption</th>
<th>No. of Cases</th>
<th>Primary Years</th>
<th>Age-Adjusted Risk (95% CI)</th>
<th>Multivariate Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 year or less</td>
<td>48</td>
<td>776</td>
<td>1.0 (Reference)</td>
<td>0.90 (0.69-1.18)</td>
<td></td>
</tr>
<tr>
<td>1-2 years</td>
<td>64</td>
<td>1232</td>
<td>0.80 (0.58-1.08)</td>
<td>0.67 (0.43-1.05)</td>
<td></td>
</tr>
<tr>
<td>&gt;2 years</td>
<td>84</td>
<td>1864</td>
<td>0.85 (0.58-1.24)</td>
<td>0.57 (0.35-0.94)</td>
<td></td>
</tr>
</tbody>
</table>

Threshold for Protective Effect for Dietary Fish Consumption

Relative Risk for Myocardial Infarction by Fish Intake

(737 Myocardial Infarctions)

Age-Adjusted Multivariate

<table>
<thead>
<tr>
<th>Fish Meals</th>
<th>RR (95%CI)</th>
<th>RR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1/mo</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1- 3/mo</td>
<td>0.94</td>
<td>0.91 (0.55-1.53)</td>
</tr>
<tr>
<td>1- &lt;2/wk</td>
<td>1.00</td>
<td>0.99 (0.64-1.54)</td>
</tr>
<tr>
<td>2- &lt;5/wk</td>
<td>1.03</td>
<td>1.03 (0.67-1.58)</td>
</tr>
<tr>
<td>5+/wk</td>
<td>1.02</td>
<td>1.00 (0.62-1.60)</td>
</tr>
</tbody>
</table>

P for Trend = 0.75 0.67

Cardiovascular Health Study

- 3910 adults aged >=65 years and free of known cardiovascular disease in 1989 and 1990.
- Provided information on consumption of tuna, other broiled or baked fish, and fried fish via a "picture sort FFQ".
- Over 9.3 years' follow-up, there were 247 IHD deaths (including 148 arrhythmic deaths) and 363 incident nonfatal myocardial infarctions.

Cardiovascular Health Study


Diet and Reinfarction Trial (DART)

Direct Evidence for n-3 Fatty Acids

**GISSI-Prevenzione Trial**

**Design**

- **Eligibility Criteria:**
  - Recent (< 3 Months) MI
  - No contraindications to supplements
  - No unfavorable short-term outlook (overt CHF, cancer)

- **Study Medications:**
  - Fish Oil (EPA/DHA 1:2): ~ 850 mg/day
  - Vitamin E: 300mg/day

- **Sample Size:**
  - 11,324 patients randomized

- **Primary Endpoint:**
  - Cumulative rate of death, non-fatal MI, and non-fatal CVA

---

**GISSI-Prevenzione Trial**

**Cause Specific Mortality**

<table>
<thead>
<tr>
<th>n-3 PUFA</th>
<th>Control</th>
<th>RR</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Death</td>
<td>136 (4.8%)</td>
<td>193 (6.8%)</td>
<td>0.80 (0.68 - 0.65)</td>
</tr>
<tr>
<td>CVD Death</td>
<td>108 (3.8%)</td>
<td>165 (5.8%)</td>
<td>0.70 (0.56 - 0.87)</td>
</tr>
<tr>
<td>CHD Death</td>
<td>100 (3.5%)</td>
<td>151 (5.3%)</td>
<td>0.66 (0.51 - 0.84)</td>
</tr>
<tr>
<td>SCD</td>
<td>55 (1.9%)</td>
<td>99 (3.9%)</td>
<td>0.55 (0.40 - 0.76)</td>
</tr>
<tr>
<td>Other Death</td>
<td>100 (3.5%)</td>
<td>100 (3.5%)</td>
<td>0.99 (0.75 - 1.30)</td>
</tr>
<tr>
<td>Non-fatal CVD</td>
<td>140 (4.9%)</td>
<td>144 (5.1%)</td>
<td>0.96 (0.76 - 1.21)</td>
</tr>
<tr>
<td>Total CVA</td>
<td>98 (1.7%)</td>
<td>80 (1.4%)</td>
<td>1.30 (0.87 - 1.96)</td>
</tr>
</tbody>
</table>

---

**Early Protection Against Sudden Death by n-3 Polyunsaturated Fatty Acids in GISSI-P**

**Blood Fatty Acid Levels and Risk of SCD as First Manifestation of CHD**

**The Physicians’ Health Study:**

**Prospective Nested Case-Control Analysis**

- 94 SCD Cases as First Manifestation of CVD.
- Baseline whole blood stored at -82°C
- 2:1 Age and Smoking Matched Controls (n = 184).
- Fatty Acids Measured by Gas-Liquid Chromatography

---

**Multivariate Relative Risk of SCD by Blood Long Chain n-3 Fatty Acid Level**

<table>
<thead>
<tr>
<th>N-3 Fatty Acid Level</th>
<th>Relative Risk</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 4.35</td>
<td>1.0</td>
<td>Referent</td>
</tr>
<tr>
<td>4.35 – &lt; 5.15</td>
<td>0.55</td>
<td>(0.18 – 1.70)</td>
</tr>
<tr>
<td>5.15 - &lt; 6.09</td>
<td>0.28</td>
<td>(0.09 – 0.87)</td>
</tr>
<tr>
<td>&gt; 6.09</td>
<td>0.19</td>
<td>(0.05 – 0.71)</td>
</tr>
</tbody>
</table>

P, trend = 0.007

* Adjusted for hypercholesterolemia, hypertension, diabetes, body mass index, family history of MI prior to age 60, vigorous exercise ≥1/wk, alcohol use, and treatment assignment
Conflicting Results on SCD
DART-2

- Randomized Trial of Fish advice and/or fish oil in 3114 men under age 70 with angina.
- Randomization over 6 years (terminated for one year)
- Two phases of Randomization: Fish advice group later sub-randomized to Fish Oil.
- Drop-out rate not specified.

Dart-2 Results

Burr ML, Eur J Clin Nut; 2003

<table>
<thead>
<tr>
<th>Dietary Fish</th>
<th>Fish oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>all deaths</td>
<td>198</td>
</tr>
<tr>
<td>Cardiac deaths</td>
<td>121</td>
</tr>
<tr>
<td>Sudden deaths</td>
<td>49</td>
</tr>
</tbody>
</table>

*Hazard ratios adjusted for age, smoking, previous MI, history of high blood pressure, diabetes, diet, serum cholesterol, medication (one type), and fruit advice.

Alpha-Linolenic Acid (ALA)

- Shorter chain n-3 fatty acid (C18:3 n-3) found in soybean, canola, flaxseed oil, nuts (primarily walnuts), and in green leafy vegetables.
- After ingestion, a small (as yet ill-defined) portion of [alpha]-linolenic acid (<10%; possibly <1%) is converted into EPA (C20:5 n-3) and DHA (C22:6 n-3).
- Conversion may be more significant in women
- ALA also appears to have antiarrhythmic effects in animal models similar to that seen with EPA and DHA
- ALA may also have direct beneficial effects on thrombosis that are not mediated through conversion to EPA and DHA

Alpha-Linolenic Acid (ALA)
Prospective Cohort Studies

- Inverse associations with CHD:
  - CHD Death
    - MRFIT
    - Nurses’ Health Study
    - Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study
  - Non-Fatal MI
    - Health Professional Follow-up Study
- No Association with CHD
  - CHD Death
  - Zutphen

Relative Risks of CHD Associated with ALA intake Stratified by Long-Chain N-3 fatty Acid Intake

Health Professionals Follow-up Study


Dietary Alpha-Linolenic Acid Intake and Risk of SCD in the Nurses’ Health Study

Dietary ALA Intake and Risk of SCD According to EPA+DHA intake

**Albert CM et al. Circulation. In Press**

n-3 Fatty Acids and AF

AF-Free Survival According to Fish Consumption in the Cardiovascular Health Study


N-3 Fatty Acids from Fish and Risk of AF: Danish Diet, Cancer, and Health Study

**Possible Mechanisms**

Epidemiologic Evidence

- Lowers Heart Rate:
  - Associations between fish intake and heart rate in observational studies
  - N-3 fatty acid supplementation lowers heart rate in small randomized trials.

- Reduces Inflammation:
  - Fish Intake and n-3 Fatty Acid levels are associated with lower levels of inflammatory markers in cross-sectional observational studies.
**Possible Mechanisms**

- **Heart rate variability:**
  - Associations between n-3 fatty acid intake and blood levels in observational studies.
  - Divergent results from small randomized trials in healthy patients versus post-MI patients with LV dysfunction.

- **PVC Frequency:**
  - Divergent results from small randomized trials in healthy patients versus post-MI patients.

- **QT Interval**
  - No effect of long chain n-3 fatty acids in a small randomized trial of healthy patients.
  - Dietary ALA intake inversely associated with QT interval duration and risk of prolonged repolarization in the NHLBI Family Heart Study.

**Electrophysiologic Effects in Humans**

- **Schrepf, et al. Lancet, 2004**
  - 10 Patients with pre-implant inducible VT and repeated episodes of VT underwent non-invasive PES
  - 7 had monomorphic sustained VT induced and received an intravenous infusion of 3.8 g n-3 PUFA
  - Of these, 5 patients were rendered non-inducible after the infusion.
  - The fish-oil infusion also prolonged the ventricular effective-refractory period.

**Summary n-3 Fatty Acids and Arrhythmias**

**Observational Data:**

- Dietary sources of n-3 fatty acids are associated with reduced risks of cardiovascular mortality in most observational studies.
- Dietary sources of long chain n-3 fatty acids and blood levels of long-chain n-3 fatty acids have been associated with reduced risks of SCD/cardiac arrest.
- Dietary sources of ALA have been associated with reduced risks of SCD in women.
- Dietary sources of long chain n-3 fatty acids have been associated both with reduced and increased risks of atrial fibrillation.

**Randomized Trial Data:**

- Dietary sources of long-chain n-3 fatty acids have been associated with reductions in CHD mortality in one trial of Post-MI patients.
- Long chain n-3 fatty acid supplementation significantly reduced SCD resulting in an overall reduction in total CHD mortality in a large randomized trial among Post-MI patients.
- Long chain n-3 fatty acid supplementation significantly reduced Post-operative atrial fibrillation in a small randomized trial among Post-CABG patients.

**Current Recommendations**

- AHA dietary recommendations now include consumption of at least two meals of fish per week.
- Fish oil supplements are not currently routinely recommended as secondary or primary prevention of SCD.
- However, patients with CHD have been advised to consume ~ 1 gram of EPA and DHA (combined) per day by the AHA.
1. Topic and Author

Ethan Balk, MD MPH and Mei Chung, MPH

3. Current challenges and the most important issues for future research

There are several areas of limitations and deficits in the current evidence from animal and in vitro models. Some are particular to the research fields of omega-3 fatty acids and arrhythmogenesis. Others are relevant to the larger research community. Among these limitations are insufficient evidence to draw conclusions; incomplete study reporting; heterogeneity of study design and measures, limiting summaries across studies; lack of standardization or consensus in study design methods, models, measures, and appropriate interventions; possible publication bias; lack of discussion of clinical meaning of findings; need for understanding of how to measure study quality.

4. Areas of overlap with other workshop topic areas

Topics/outcomes summarized (through 2003):
- Animal models of arrhythmia (VTach, VFib, VPB, etc.)
- Isolated organ and cell models
  - Arrhythmias
  - Basoelectromechanical parameters
  - Ion currents

6. Citations

2. www.ahrq.gov/clinic/epcindex.htm
3. www.ahrq.gov/clinic/tp/o3arrtp.htm
Evidence Report: Effects of n-3 FA on Arrhythmogenic Mechanisms (Animal / In Vitro Studies)

Ethan Balk, MD MPH
Associate Director
ebalk@tufts-nemc.org

Mei Chung, MPH
Research Associate
mchung1@tufts-nemc.org

Tufts-New England Medical Center Evidence-based Practice Center
Boston, MA

Joseph Lau, MD
Director, Tufts-NEMC EPC
jlau1@tufts-nemc.org

Alice Lichtenstein, DSc
Director of the Cardiovascular Nutrition Laboratory
USDA Human Nutrition Research Center on Aging
alice.lichtenstein@tufts.edu

Animal / In vitro Studies

• 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 that omega-3 fatty acids directly affect cell organelles such as cardiac ion channels, pumps, or exchange mechanisms involved in electrogenesis?

Steps to Perform a Systematic Review

FORMULATE STUDY QUESTION
ESTABLISH PROTOCOL
LITERATURE SEARCH - RETRIEVAL
PAPER SELECTION per PROTOCOL
DATA EXTRACTION
CRITICAL APPRAISAL
QUALITY ASSESSMENT
ANALYSIS & INTERPRETATION

Whole Animal Studies (n=26)

• Outcomes:
  – Ventricular fibrillation (19)
  – Ventricular fibrillation threshold (4)
  – Ventricular tachycardia (13)
  – Ventricular premature beats (13)
  – Arrhythmia score (10)
  – T5f: time in normal sinus rhythm (5)
  – Infarct size, death
• n-3 Feeding (23) and Infusion (3)
  – Esterified DHA, EPA, eEPA, Fish oil (various)
  – ALA, Linseed oil, Soybean oil
• Controls
  – n-6 (15), MUFA (1), SFA (5), Chow (5)
• Models
  – Rat (14), Dog (7), Monkey (3), Rabbit (1), Pig (1)

Methods

• Formulate questions (PICO)
  – Population, Interventions, Comparators, Outcomes
• Literature search strategy
  – Multiple databases searched
  – Domain experts, References
• Eligibility criteria
  – English
  – Evaluate impact of n-3 on arrhythmia, intermediate mechanisms of arrhythmia, and electrogenesis
  – Exclude letters, abstracts, posters
• Summarize results

Literature Search Results

(April 2003)

• Abstracts screened 1807
• Papers retrieved & screened 274
• Articles included
  – Whole animal 26
  – Whole-animal isolated organs and cells 21
  – Isolated organs and cell cultures 39
Selected Results (meta-analyses)

• VTach (ischemia induced, rats, n-6 control)
  – N=4, fed ALA, 0.4-5.2g/100g, n=112
    RR = 0.82 (0.65-1.00)
  – N=6, fed EPA+DHA, 2.1-3.7g/100g, n=136
    RR = 0.49 (0.29-0.83)

• VTach (reperfusion induced, rats, n-6 control)
  – N=5, fed ALA, 0.4-1.2g/100g, n=125
    RR = 1.1 (0.73-1.6)
  – N=6, fed EPA+DHA, 2.6-3.7g/100g, n=132
    RR = 0.68 (0.50-0.91)

• VFib (ischemia induced, rats, n-6 control)
  – N=3, fed ALA, 1.1-5.2g/100g, n=76
    RR = 0.95 (0.56-1.6)
  – N=5, fed EPA+DHA, 2.1-3.7g/100g, n=100
    RR = 0.21 (0.07-0.63)

• VFib (reperfusion induced, rats, n-6 control)
  – N=6, fed ALA, 0.4-5.2g/100g, n=144
    RR = 0.84 (0.52-1.3)
  – N=8, fed EPA+DHA, 1.2-3.7g/100g, n=168
    RR = 0.44 (0.25-0.79)

Summary

Rat Study Meta-Analyses

<table>
<thead>
<tr>
<th></th>
<th>VTach Isch</th>
<th>VTach Reper</th>
<th>VFib Isch</th>
<th>VFib Reper</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EPA/DHA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Overall ALA

• 6 studies (4 feeding, 2 infusion)

<table>
<thead>
<tr>
<th></th>
<th>VTach Isch</th>
<th>VTach Reper</th>
<th>VFib Isch</th>
<th>VFib Reper</th>
</tr>
</thead>
<tbody>
<tr>
<td>VTach (ischemia induced)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>VTach (reperfusion induced)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VFib (ischemia induced)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>VFib (reperfusion induced)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arrhythmia Score</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>V Premature Beats</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Time in Sinus Rhythm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Overall EPA/DHA

• 25 studies (22 feeding, 3 infusion)

<table>
<thead>
<tr>
<th></th>
<th>VTach Isch</th>
<th>VTach Reper</th>
<th>VFib Isch</th>
<th>VFib Reper</th>
</tr>
</thead>
<tbody>
<tr>
<td>VTach (ischemia induced)</td>
<td>9</td>
<td>3</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>VTach (reperfusion induced)</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>VFib (ischemia induced)</td>
<td>11</td>
<td>5</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>VFib (reperfusion induced)</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>VFib Threshold</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Arrhythmia Score</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>V Premature Beats</td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Time in Sinus Rhythm</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Conclusion (Animal Studies)

• Fish oil supplementation may have antiarrhythmic effects
  – Ability to reduce ventricular tachycardia and ventricular fibrillation in ischemia-induced arrhythmia models
  – Other measures, including reperfusion-induced arrhythmias, are inconclusive overall
    • Subset of rat studies support benefit for reperfusion-induced arrhythmias, by meta-analysis
  – No significant or consistent benefit was observed with ALA supplementation
Whole Animal (Fed) Isolated Organ and Cell Studies

- Outcomes:
  - Basoelectromechanical parameters (3)
  - Ion currents (2)
  - (contractile parameters, ATPase activity, ion movement, ion channels)
- All fish oil
- Controls
  - High fat diets, Safflower oil
- Models
  - Fed rats, Fed rabbits

Results

- Basoelectromechanical parameters
  - Fed Rats: FO significantly ↓ VERP
  - Perfused rat hearts: FO → No Δ
  - Fed Rabbits: FO → No Δ
- Ion currents
  - (Fed rats, ventricular myocytes)
    - $I_{Na}$: FO → No Δ activation / inactivation
    - $I_{to}$: FO → No Δ activation / inactivation
    - $I_{Na}$: FO → No Δ activation / inactivation / amplitude

Isolated Organ and Cell Studies Perfused / Incubated

- Outcomes:
  - Arrhythmias (7)
  - Basoelectromechanical parameters (9)
  - Ion currents (12)
  - (contractility, inotropy, ion movement, ion channels)
- All fish oil, 3 also ALA
- Models
  - rats, ferrets, rabbits, mice, guinea pigs, cat

Arrhythmia and Basoelectromechanical Parameters

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>0</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asynchronous contractions: Free E/D</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bound E/D</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ALA</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Action potential</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Action potential amplitude (APA)</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>APD</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>APD</td>
<td>3.5*</td>
<td>2</td>
<td>0.5*</td>
</tr>
<tr>
<td>Max rate depolarization ($V_{max}$)</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Max diastolic potential (MDP)</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Overshoot potential</td>
<td>2.5*</td>
<td>0</td>
<td>0.5*</td>
</tr>
<tr>
<td>* Increase or decrease in different conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ion Currents

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{Na}$</td>
<td>3</td>
<td>2 ↓ , contradictory activation / inactivation</td>
</tr>
<tr>
<td>$I_{to}$</td>
<td>4</td>
<td>1 ↑ / 3 ↓ amplitude</td>
</tr>
<tr>
<td>$I_{Ca-L}$</td>
<td>6</td>
<td>4 ↓ voltage</td>
</tr>
<tr>
<td>$I_{K}$</td>
<td>2</td>
<td>↓ current</td>
</tr>
<tr>
<td>$I_{KI}$</td>
<td>4</td>
<td>1 ↓ current / 3 no effect</td>
</tr>
<tr>
<td>$I_{KUR}$</td>
<td>2</td>
<td>↓ current with higher n-3 concentrations</td>
</tr>
</tbody>
</table>

Conclusion (Organ / Cell Studies)

- Basoelectromechanical Parameters
  - Small number of studies, large heterogeneity of study designs and parameters measured
  - Heterogeneity of results
  - Could not conclude that any definitive effect
- Arrhythmia
  - n-3 FA (7 EPA/DHA, 1 ALA) has a protective effect against spontaneous or induced arrhythmias
- Ion Currents
  - Small number of studies
  - Possibly sufficient evidence that n-3 FA decreases voltage dependent L-type Ca current ($I_{Ca-L}$)
  - Possibly sufficient evidence that n-3 FA has no effect on inward rectifier potassium current ($I_{KI}$)
Limitations

- Reporting often incomplete
- Narrow range of sources of studies
  - 70% of animal n-3 studies from 1 lab
- Heterogeneity results in difficulty summarizing (lack of standardization, consensus about appropriate models)
- Lack of consensus regarding appropriate form of n-3 fatty acids or appropriate dose
- Lack of consensus about appropriate controls
  - ? n-6 or MUFA best
- Lack of consensus about appropriate models
  - E.g., ischemic vs. arrhythmogenic models
- Intervention mode (fed, infused)
  - Adds to heterogeneity of studies
  - Studies rarely discuss how intervention mode may affect results
- Animal models
  - Rat 60
  - Dog 10
  - Guinea pig 4
  - Mouse 4
  - Monkey 3
  - Rabbit 3
  - Pig 2
  - Ferret 1
  - Cat 1
- Reporting of animals, conditions, and diets
  - Generally very minimal beyond strain and age
  - Animal source, sex, body weight, housing condition (stress factors), diet, season
  - All items that can confound analysis
- Investigator blinding and subject randomization
  - Basic standards of human studies are lacking in basic science studies
  - Unclear what is the effect of lack of blinding/random
- Publication bias
  - All animal and in vitro studies for omega-3 fatty acids reported positive effects
  - Null or negative effects reported only in conjunction with positive effects
  - “Primary outcome” almost always positive
- Statistical v Clinical effect / Lab v Biological effect
  - Little discussion regarding whether the statistically significant findings are biologically meaningful
  - Little discussion regarding how lab findings may correlate with human disease/health
- Research needed on how to evaluate quality
Session II

NHLBI-supported Trials to Determine the Antiarrhythmic Effects of n-3 Fatty Acids
1. Topic and Author

Results of Fatty Acid Antiarrhythmic Trial (FAAT) – (R01 HL062154)
Alexander Leaf, M.D.

2. Where we stand in 2005.

In my clinical trial, which has been accepted for publication in Circulation, we report that the fish oil n-3 fatty acids proved to be very potent antiarrhythmic agents, preventing fatal ventricular arrhythmias in high risk patients with implantable cardioverter defibrillators (ICDs). We report a reduction of 48% (P=0.0060) in 236 enrollees in our trial, who continued to take their prescribed fish oil capsules (2.4 g of EPA+DHA) daily for their full year in the study.

3. Current challenges and the most important issues for future research

I think the paradox between our beneficial effects of the n-3 fish oil fatty acids on high risk patients with ICDs and the contrary findings reported by Dr. McAnulty, will be a very important issue for future research.

I have some ideas which I hope there will be time to illustrate and explain from the research by my group has done on the mechanism of the antiarrhythmic action of these interesting fish oil fatty acids.

6. Citations

Prevention of Sudden Cardiac Death

Alexander Leaf, MD
Harvard Medical School

Table 3. Analysis of Time to First Event.

<table>
<thead>
<tr>
<th>Analysis Type</th>
<th>Number</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intention to Treat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Confirmed&quot; events</td>
<td>0.72</td>
<td>0.50 - 1.03</td>
<td>0.067</td>
</tr>
<tr>
<td>Including &quot;Probable&quot;</td>
<td>0.69</td>
<td>0.49 - 0.95</td>
<td>0.031</td>
</tr>
<tr>
<td>Multivariate Analysis*</td>
<td>0.67</td>
<td>0.47 - 0.95</td>
<td>0.034</td>
</tr>
<tr>
<td>On-Treatment Analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Confirmed&quot; events</td>
<td>0.67</td>
<td>0.46 - 0.94</td>
<td>0.024</td>
</tr>
<tr>
<td>Including &quot;Probable&quot;</td>
<td>0.66</td>
<td>0.46 - 0.92</td>
<td>0.016</td>
</tr>
<tr>
<td>Multivariate Analysis*</td>
<td>0.65</td>
<td>0.45 - 0.92</td>
<td>0.026</td>
</tr>
</tbody>
</table>

*Multivariate model controlled for gender, left ventricular ejection fraction (continuous), NYHA Class III congestive heart failure, history of myocardial infarction, history of prior defibrillator therapies for VT/VF, and baseline left ventricular ejection fraction.

**On-treatment analysis for all subjects who had taken any of their prescribed oil supplements; the follow-up was censored at two months after stopping medication.

***On-treatment only of those subjects who were on treatment at least 11 months.
Antiarrhythmic Polyunsaturated Fatty Acids
Collaborating Colleagues

Jing X Kang
Yong-Fu Xiao
George E. Billman
Yunyuan Li
Ana Maria Gomez
W. Jon Lederer
James P. Morgan

Konstantin Bogdanov
Salvatore Pepe
Edward Lakatta
Haifa Hallaq
Thomas W. Smith
Alois Sellmayer
Robert Voskayi
Martin Vreugdenhil
Claus Braehl
Wytse J. Wadman
1. Topic and Author

Results of Antiarrhythmic Effects of n-3 Fatty Acids Study (R01 HL061682)
John McAnulty, M.D.

2. Where we stand in 2005.

Results from this and other studies will have to be assessed to address apparent discrepancies in outcomes.

Summary: To test the hypothesis that n-3 polyunsaturated fatty acids (n-3 PUFA) have antiarrhythmic properties in humans, we performed a prospective, double-blinded, randomized, placebo controlled trial of fish oil supplementation in patients with a recent episode of a primary sustained ventricular arrhythmia who received, or had received an implantable defibrillator (ICD).

Results: DHA and EPA levels in RBC membranes and plasma rose and were consistently higher in the 100 fish oil patients than in the 100 placebo patients (p <0.0001). The time to the first episode of ICD therapy for VT or VF after randomization, the 1° end point of the study, did not differ between the 2 treatment groups (p = 0.19). Fish oil may have been proarrhythmic in patients who had received the ICD for primary VT.

3. Current challenges and the most important issues for future Research

   a) Refine mechanisms of n-3 PUFA still further
   b) Assess interacting variables-ischemia, ventricular function, drug-drug interactions, etc.
   c) Define phenotypic and genotypic profiles of population most likely to benefit (or be harmed) by n-3 PUFA intake

4. Areas of overlap with other workshop topic areas

The results of this (and other) evaluation(s) in humans may be explained in part by the mechanisms to be discussed.

5. Translating the topic into understanding effects of n3-fatty acids on arrhythmogenesis

The difference in outcome in those presenting with clinical VF versus clinical VT would seem
most likely due to differences in mechanism/drug affect interaction.

6. Citations

Antiarrhythmic Effects of n-3 Polyunsaturated Fatty Acids

Merritt Raitt MD, William Connor MD, Cynthia Morris PhD, Jack Kron MD, Blair Halperin MD, Sumeet Chugh MD, James McClelland MD, James Cook MD, Karen MacMurdy MD, Robert Swenson MD, Sonja Connor LD, Glenn Gerhard MD, Daniel Oseran MD, Christy Marchant RN, David Calhoun RN, Reed Snyder MD, John McAnulty MD

n-3 Polyunsaturated Fatty Acids (ω-3 fatty acids)

- Essential fatty acids
- Dietary sources include cold water fish (fish oil), flaxseed oil, walnut oil, and canola oil
- EPA: C20:5n-3 Eicosapentaenoic
- DHA: C22:6n-3 Docosahexaenoic

Evidence ω-3 Fatty Acids are Antiarrhythmic

- Observational and Case Control Studies
  - High fish intake and high blood ω-3 fatty acid levels associated with a reduced risk of SCD
  - SCD victims have low ω-3 fatty acid levels
- Basic Science and Animal Models
  - ω-3 fatty acids prevent ischemic VF
  - ω-3 fatty acids inactivate Na+ channels (Class I)
- 3 Randomized Clinical Trials in Humans after MI
  - Reduced risk of sudden death
  - No change in risk MI

Physicians Health Study

- ≥1 fish meal per week
  - Relative risk of SCD = 0.44 (0.22-0.86, p=0.006) corrected for known risks
  - No association with risk of MI
- Highest quartile of ω-3 fatty acid in blood
  - 6-10% of whole blood fatty acid
  - Lowest relative risk of SCD = 0.10 (0.02-0.48, p=0.001) corrected for known risks


ω-3 Fatty Acids Prevent Ischemic VF in Animal Models

- Rat - long term feeding studies
  - ω-3 fatty acids: ischemic VF reduced 43%
  - Olive oil: no effect
- Dog - acute infusion
  - ω-3 fatty acids: reduced ischemic VF 75%
  - EPA and DHA both effective

McLennan et al Can J Physiol Pharmacol 1985;63:1411-1417
Billman et al Circulation 1999;99:2452-2457

GISSI Prevenzione Trial - Methods

- Multicenter, open label, prospective, randomized trial
- 11,323 patients < 3 months after MI
- 1 gram fish oil (EPA + DHA) or placebo daily
GISSI Prevenzione Trial - Results

Hypothesis

• Supplementation with ω-3 fatty acids will reduce the incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF) in patients with implantable defibrillators (ICDs) who have had a recent episode of VT or VF

Study Design

• Multi-center, double blinded, randomized, placebo controlled trial of fish oil supplementation in 200 patients with ICDs and a recent episode of VT or VF.

Entry Criteria

• New ICD implant for sustained VT or VF
  OR
• Therapy for VT or VF within the last 3 months from an existing ICD

Exclusion Criteria

• Class I or Class III antiarrhythmic therapy
• >1 fatty fish meal per week

Methods

• Fish oil
  • 1.8 grams daily, 42 % EPA, 30 % DHA
  • 2 capsules BID
• Placebo
  • olive oil, 2 capsules BID
• All patients counseled to follow an AHA step 1 diet (30% of calories from fat).
• Patients followed for 2 years
• Episodes of ICD therapy adjudicated by a blinded committee

End Points

• Primary
  • Time to first episode of VT or VF
• Secondary
  • Time to first VT or VF in subgroups
  • Time to recurrent episodes of VT or VF
  • Correlation between ω-3 fatty acids and time to VT or VF
  • Electrophysiologic changes due to ω-3 fatty acids
### Baseline Demographics

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (n=100)</th>
<th>Placebo (n=100)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63±13</td>
<td>62±13</td>
<td>0.40</td>
</tr>
<tr>
<td>Male</td>
<td>86%</td>
<td>86%</td>
<td>1.00</td>
</tr>
<tr>
<td>VT at entry</td>
<td>64%</td>
<td>69%</td>
<td>0.55</td>
</tr>
<tr>
<td>VF at entry</td>
<td>36%</td>
<td>31%</td>
<td>0.55</td>
</tr>
<tr>
<td>CAD</td>
<td>75%</td>
<td>71%</td>
<td>0.63</td>
</tr>
<tr>
<td>MI</td>
<td>55%</td>
<td>56%</td>
<td>1.00</td>
</tr>
<tr>
<td>Ejection Fraction</td>
<td>0.36±0.16</td>
<td>0.35±0.15</td>
<td>0.60</td>
</tr>
</tbody>
</table>

### Red Cell Membrane DHA + EPA

- **% DHA + EPA**
  - Fish oil
  - Placebo
  - p < 0.0001

### Time to First VT/VF

- **% without VT or VF**
  - Placebo
  - Fish oil
  - p = 0.19

### Time to First VT/VF in Patients Enrolled after VT

- **% without VT or VF**
  - Placebo
  - Fish oil
  - p = 0.007

### Time to First VT/VF in Patients with VF at Entry

- **% without VT or VF**
  - Placebo
  - Fish oil
  - p = 0.38

### Time to VT VF by RBC ω-3 Fatty Acid Levels

- **% without VT or VF**
  - ω-3 quartiles 1-3
  - ω-3 quartile 4
  - p = 0.39
Conclusions

- ω-3 fatty acids do not have antiarrhythmic effects in survivors of ventricular tachyarrhythmias
- Fish oil supplementation
  - No difference in time to first VT/VF
  - Increased risk of VT/VF in patients with prior VT
  - Increased risk of recurrent episodes of VT/VF

Acknowledgements

- Participating hospitals
  - Oregon Health and Sciences University, Portland OR;
  - Portland VA Medical Center, Portland, OR;
  - St Vincent Medical Center, Portland, OR;
  - Oregon Cardiology PC, Eugene, OR;
  - Baystate Medical Center, Springfield, MA;
  - Southwest Washington Medical Center - Vancouver WA
- OHSU CRC
- F. Hoffman-La Roche Ltd
- Data safety and monitoring board
- NIH RO1 HL61682-03
Session III

Possible Basic Mechanisms of Action
1. **Topic and Author**

**Dietary Sources of n-3 Fatty Acids: Metabolic Pathways & Sites of Interaction**  
Bill Lands, Ph.D.

2. **Where we stand in 2005.**

In 2005, competitions among the various n-3 and n-6 fatty acids are recognized to occur during metabolic processes in tissues, especially with 20- and 22-carbon highly unsaturated fatty acids (HUFA). However, proportions of tissue HUFA are often inadequately monitored, quantitated or documented in published clinical studies, and many study designs use dietary changes insufficient to appreciably alter tissue proportions and give appreciable physiological effects. The inadequate monitoring and reporting of tissue status gives many clinical reports that focus on a limited aspect of the competing tissue components, limiting the context of the published observations and interpretations in ways that prevent readers from evaluating alternative explanations.

3. **Current challenges and the most important issues for future research**

Researchers should obtain values of biomarkers that indicate the competing proportions of n-3 and n-6 acids in tissues to ensure adequate dietary interventions and avoid giving results in a too-limited context. When tissue data are not available, dietary information should be complete enough to estimate quantitatively the degree to which the omega-3 intervention being studied has affected the tissues.

4. **Areas of overlap with other workshop topic areas**

The status of the tissue membrane lipids (that are released during tissue responses to stress) likely overlaps with all evaluations of tissue response in this workshop.

5. **Translating the topic into understanding effects of n3-fatty acids on arrhythmogenesis**

Arrhythmogenesis is a response to various factors that alter heart rhythm, and it needs to be interpreted in a broad context of etiological factors that include vagal sympathetic and parasympathetic tone (HRV), oxygen supply (ischemic and thrombotic events), tissue HUFA proportions and endothelial mediators. Designating all "sudden death" as being caused by arrhythmia fails to recognize the multiple etiological pathways to arrhythmia and death, each of which can be influenced by the tissue proportions of n-3.
and n-6 HUFA. Effects attributed to higher intakes of n-3 HUFA may be caused by a lower availability of n-6 HUFA-derived autacoids at tissue receptors.

6. Citations

Dietary Sources of n-3 Fatty Acids: Metabolic Pathways & Sites of Interaction

**Dietary Lipids**
- Plasma Lipoproteins
- Membrane Phospholipids
- Triglycerides
- Diglycerides
- NEFA
- Acyl-CoA
- HUFA
- Elongation & Desaturation
- (n-3) (n-6)

**Strategic Sites**
- Lipoprotein Lipase
- NEFA
- Acyl-CoA
- HUFA
- Elongation & Desaturation
- (n-3) (n-6)

**Dietary Sources of n-3 Fatty Acids**
- 18:2n-6 & 18:3n-3 are maintained in triglycerides linearly with dietary 18:2 & 18:3
- 20:3+20:4n-6 & 20:5+22:5n-3 are maintained in phospholipids hyperbolically with dietary 18:2 & 18:3
Very low intakes of n-3 or n-6 acids prevent EFA deficiency
Mohrhauser & Holman J. Lipid Res. 3: 151-159, 1963
(http://efaeducation.nih.gov/sig/dri.html)

Hyperbolic metabolic conversion of dietary PUFA to tissue HUFA

Dietary LA and serum tetraenes
Hansen et al. 1963 Pediatrics 31: 171-192

Dietary LA and serum trienes
Hansen et al. 1963 Pediatrics 31: 171-192

Tissue HUFA are maintained by dietary PUFA
an empirical hyperbolic metabolic relationship

\[
\begin{align*}
20:3&+20:4n-6 \\
in 
\text{HUFA} & = 100 \\
\text{HC}_3 & = 3.0 \\
\text{HC}_6 & = 0.70 \\
\text{PC}_3 & = 0.0555 \\
\text{PC}_6 & = 0.0441 \\
\text{C}_6 & = 5.0 \\
\text{K}_a & = 0.175 \\
\end{align*}
\]

Lands et al, BBA 1180: 147-162 (1992)
[constants revised in 2002]

A handy calculator for planning trials is at:
http://efaeducation.nih.gov/dietbalance.html

An equation predicting HUFA dyslipidemia is at:
http://efaeducation.nih.gov/hufacalc.html

Diet predicts HUFA proportions in plasma

<table>
<thead>
<tr>
<th>Diet</th>
<th>Typical diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>US</td>
<td>0.80, 0.85, 0.60, 0.76 (short 3)</td>
</tr>
<tr>
<td>Amed/Jap</td>
<td>1.50, 0.62, 2.30, 0.54 (short 6)</td>
</tr>
<tr>
<td></td>
<td>0.03, 0.09, 0.54 (long 3)</td>
</tr>
<tr>
<td></td>
<td>0.08, 0.08, 0.08 (long 6)</td>
</tr>
</tbody>
</table>

AverAge Daily Dietary intakes

<table>
<thead>
<tr>
<th>n-3 HUFA</th>
<th>n-6 HUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.80</td>
<td>0.08</td>
</tr>
<tr>
<td>0.85</td>
<td>0.08</td>
</tr>
<tr>
<td>0.60</td>
<td>0.08</td>
</tr>
<tr>
<td>0.76</td>
<td>0.08</td>
</tr>
</tbody>
</table>

% n-6 in HUFA

42, 80, 62, 51

An equation predicting HUFA dyslipidemia is at:
http://efaeducation.nih.gov/hufacalc.html

Overall Predicted & Observed HUFA Proportions

% n-3 HUFA

140 CHD/100,000
93 CHD/100,000
66 CHD/100,000

Quebec 18-34 35-49 50+ 18-34 35-49 50+ 18-34 35-49 50+
Cree 30 19 13 21 21 21 7 35 39 25
Inuit 35 13 3 32 13 21 20 25 44 10
HUFA imbalance is a diet-induced dyslipidemia

CHD Mortality and Tissue HUFA

\[ y = 3.0323 \times n - 74.8 \]
\[ R^2 = 0.9866 \]

% n-6 HUFA in Total HUFA

<table>
<thead>
<tr>
<th>% n-6 HUFA in Total HUFA</th>
<th>CHD Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Quebec Cree</td>
</tr>
<tr>
<td>50</td>
<td>Quebec Urban</td>
</tr>
<tr>
<td>80</td>
<td>USA</td>
</tr>
<tr>
<td>10</td>
<td>Greenland</td>
</tr>
<tr>
<td>20</td>
<td>Japanese</td>
</tr>
</tbody>
</table>

Diet-induced Dyslipemias and Disease

Heart damage starts in childhood

Likely Mortality Risk from HUFA Dyslipemia

(Deaths per 100,000 population)

based on CHD deaths per 100,000 = 3 x (% n-6 HUFA in HUFA) - 75 & diet-tissue pattern at http://efaeducation.nih.gov/sig/hufacalc.html
Keep the calories under control
Balance the eicosanoid precursors in your body
Other facts on your foods

Distant learning websites for essential fatty acids and eicosanoids
http://ods.od.nih.gov/eicosanoids/
http://efaeducation.nih.gov/

Plan daily menus that balance tissue HUFA using interactive software, KIM-2

Dietary EFA have a role in disease and death

A handy calculator for planning trials is at:
http://efaeducation.nih.gov/dietbalance.html

An equation predicting HUFA dyslipidemia is at:
http://efaeducation.nih.gov/hufacalc.html

**DIET PREDICTS HUFA PROPORTIONS IN PLASMA**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Typical diets</th>
<th>USA</th>
<th>Med</th>
<th>Japan</th>
</tr>
</thead>
<tbody>
<tr>
<td>en% 18:3n-3</td>
<td>0.76</td>
<td>0.85</td>
<td>0.60</td>
<td>0.76 (short 3)</td>
</tr>
<tr>
<td>en% 18:2n-6</td>
<td>1.00</td>
<td>6.82</td>
<td>2.30</td>
<td>5.04 (short 6)</td>
</tr>
<tr>
<td>en% n-3 HUFA</td>
<td>0.20</td>
<td>0.03</td>
<td>0.09</td>
<td>0.54 (long 3)</td>
</tr>
<tr>
<td>en% n-6 HUFA</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08 (long 6)</td>
</tr>
</tbody>
</table>

**PREDICTED % n-6 in HUFA**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35</td>
<td>80</td>
<td>62</td>
<td>51</td>
</tr>
</tbody>
</table>
1. Topic and Author

Role of Calcium-Calmodulin Interactions in Arrhythmogenesis: Possible Sites of n-3 Fatty Acid Modulation
Mark E Anderson, M.D., Ph.D.

2. Where we stand in 2005.

Omega-3 FFA can affect a broad range of cellular Ca2+ homeostatic proteins. These actions are generally consistent with antiarrhythmic actions for suppressing afterdepolarizations and cellular Ca2+ oscillations. On the other hand, omega-3 FFA also inhibit protein kinase A and calmodulin kinase II and reduce oxidant stress in a manner that could directly or indirectly alter the activity of some Ca2+ dependent signaling molecules and arrhythmias.

3. Current challenges and the most important issues for future Research

Critical gaps in the literature include lack of adequate in vivo models, potential species-specific actions of omega-3 FFA and uncertainty about the relationship between acute effects of 'pharmacological' versus chronic effects of dietary omega-3 FFA on arrhythmia mechanisms and uncertainty regarding the key molecular constituents of fish oil that specifically affect arrhythmia mechanisms.

4. Areas of overlap with other workshop topic areas

There is potential overlap with Dr. Billman (acute effects of n-3 fatty acids in large animal models) and Dr. Giles (possible sites of n-3 fatty acid actions on electromechanical activity).

6. Citations

4. Rodrigo GC, Dhanapala S, Macknight AD. Effects of eicosapentaenoic acid on the contraction of intact, and spontaneous contraction of chemically permeabilized
2. Banthi et al BBRC 2005
4. Xia et al BBRC 2004
Fish oil, calcium and arrhythmias: is there a connection?

Mark E Anderson, MD, PhD
Betty & Jack Bailey Professor of Cardiovascular Medicine

Overview

Fish oil is a Ca^{2+} antagonist
Fish oil inhibits PKA and calmodulin kinase activity
Fish oil inhibits oxidant stress

Fish oil (EPA) is a Ca^{2+} antagonist

Fish oil (EPA) slows spontaneous contractions & reduces intracellular Ca^{2+}

Interspecies differences in mechanical responses to fish oil (EPA)

Fish oil (DHA) reduces cellular Ca^{2+} responses to depolarization, endothelin & hypoxia
Fish oil is a Ca\(^{2+}\) antagonist

Where does it work?

**Ca\(^{2+}\) entry**

L-type Ca\(^{2+}\) current

T-type Ca\(^{2+}\) current

Adrenal cells

Rodrigo et al, JMCC 1999

Banthi et al, BBRC 2005

**Fish oil (DHA) antagonizes dihydropyridine actions**

Adult rat cardiomyocytes

Fish oil (EPA) reduces reverse mode I\(_{Na/Ca}\) ex

HEK293

Xiao et al, BBRC 2004

**Ca\(^{2+}\) removal**

Fish oil (EPA) increases SR Ca\(^{2+}\) content but not the rate of Ca\(^{2+}\) egress via the Na/Ca exchanger

Adult rat cardiomyocytes

Swan et al, Cardiovasc Res 2003

O'Hall et al, J Physiol 2002

**SR Ca\(^{2+}\) release**

Fish oil (EPA) reduces RyR Po and Ca\(^{2+}\) spark parameters

Sheep SR vesicles

Adult rat cardiomyocytes

Hohnen et al, J Mem Br 2003
Fish oil feeding for 3 weeks reduces isoproterenol induced ‘arrhythmia’ but not SR Ca\(^{2+}\) content

**Conclusions 1**

Fish oil significantly reorders Ca\(^{2+}\) homeostasis in heart

Fish oil actions on cellular Ca\(^{2+}\) appear to correlate with suppression of cellular arrhythmia surrogates

Dietary and in vitro fish oil are both ‘antiarrhythmic’ but have differing actions on SR Ca\(^{2+}\)

Species differences may be important for fish oil effects on Ca\(^{2+}\) homeostasis

**Is Ca\(^{2+}\) the whole story?**

- Membrane fluidity
- Protein kinases
- Oxidation
- Inflammation

**Fish oil (EPA & DHA) inhibit PKA and CaMKII**

**PKA and CaMKII are part of a common signaling pathway**

- Low cytoplasmic Ca\(^{2+}\)
- High Ca\(^{2+}\) Inside SR

**CaMKII is a signal for arrhythmias & structural heart disease**

<table>
<thead>
<tr>
<th>CaMKII activity/expression</th>
<th>CaMKII activity may link neurohumoral activation with adverse remodeling &amp; arrhythmias</th>
</tr>
</thead>
<tbody>
<tr>
<td>In structural heart disease</td>
<td>CaMKII is recruited by (AR)cellular Ca mobilization</td>
</tr>
<tr>
<td>Patients</td>
<td>Kirchhefer Cardiov 1999</td>
</tr>
<tr>
<td></td>
<td>Kirchhefer Circ Res 1999</td>
</tr>
<tr>
<td></td>
<td>Wu Circ 2002</td>
</tr>
<tr>
<td>Animal models</td>
<td>CaMKII over-expression causes adverse remodeling</td>
</tr>
<tr>
<td></td>
<td>Zhang T Circ Res 2003</td>
</tr>
<tr>
<td></td>
<td>Zhang T Circ Res 2003</td>
</tr>
<tr>
<td></td>
<td>CaMKII inhibition suppresses adverse remodeling</td>
</tr>
<tr>
<td></td>
<td>Zhang T Nat Med 2005</td>
</tr>
<tr>
<td>Review</td>
<td>CaMKII is proarrhythmic</td>
</tr>
<tr>
<td></td>
<td>Zhang T Cardiov Rev 2004</td>
</tr>
</tbody>
</table>

Zhang et al Nat Med 2005
PKA, CaMKII & fish oil share Ca\textsuperscript{2+} homeostatic protein targets

Conclusions 2

Fish oil can inhibit protein kinases by an unknown, ATP binding-independent mechanism

Fish oil, PKA & CaMKII act at overlapping Ca\textsuperscript{2+} homeostatic proteins

Suppression of Ca\textsuperscript{2+}-dependent arrhythmias by fish oil is consistent with PKA and CaMKII inhibition

Fish oil inhibits oxidant stress in vivo

Nrf2 significantly determines oxidant stress reserve

Nrf2 is up-regulated by fish oil

Nrf2 is nearly absent after MI
Conclusions 3

Dietary fish oil can reduce myocardial oxidant stress

Up-regulation of Nrf2 activity is an appealing mechanism for increased oxidant stress reserve by fish oil

Conclusions 4

By influencing diverse signaling processes, antiarrhythmic actions of fish oil may extend beyond mechanisms immediately & directly linked to cellular Ca^2+

Limitations to current knowledge

Relative lack of in vivo data

Uncertain relationship between in vitro and in vivo data

Mostly acute experiments

Correlation between in vitro and in vivo dosing?

Interspecies variation

Molecular structure-function: identity of critical constituents, oxidation status, mechanisms of antagonist actions
Anti-inflammatory mechanisms of the anti-arrhythmic effects of n-3 fatty acids
David R. Van Wagoner, Ph.D.

Lethal arrhythmias (AF or VF) involve both arrhythmia triggers and a tissue substrate amenable to reentrant activity. In the absence of triggers, re-entrant activity is not initiated. In the absence of a suitable substrate, ectopic triggers provoke merely premature atrial or ventricular contractions. Studies from our group have revealed a strong association between a marker of systemic inflammation (C-reactive protein, CRP) and the persistence of atrial fibrillation (AF)\textsuperscript{1,2}. The natural history of AF typically involves a progression from premature atrial contractions, to paroxysmal episodes of AF, followed by more persistent episodes. Increased arrhythmia persistence is due to the combined influences of electrical remodeling (changes in ion channel expression and/or function) and structural remodeling (infarction; myocyte necrosis, apoptosis; fibroblast proliferation; interstitial matrix accumulation). Studies in experimental animal models and in patients suggest that the expression of cardiac ion channels is relatively dynamic, with the effective refractory period returning to baseline levels within 48 hours, following 5 days of AF or high-rate atrial pacing\textsuperscript{3}. Structural changes, including those identified above, are implicated in the progressive changes in fibroblast density and extracellular matrix deposition. Because of the greater plasma concentration of CRP in patients with persistent than paroxysmal AF, we hypothesized that the inflammatory response might reflect ongoing structural changes that lead to the increased persistence of AF\textsuperscript{2}. Recent studies in animal models\textsuperscript{4,5} suggest that corticosteroids such as methyl-prednisone can lower systemic CRP levels and decrease AF inducibility. Intriguingly, a recent study suggests that methyl-prednisone can lower systemic CRP levels and decrease the recurrence of AF in patients treated at first presentation\textsuperscript{6}. While exciting as a proof-of-concept, lifelong therapy with steroids is neither feasible nor desirable, due to the numerous and significant side effects\textsuperscript{7}. The anti-inflammatory actions of dietary and/or supplemental n-3 fatty acids may offer an attractive alternative to steroid therapy, and their anti-inflammatory actions are likely to contribute to the anti-arrhythmic efficacy of this therapeutic approach.

AF following cardiac surgery is strongly associated with the systemic inflammatory response\textsuperscript{8}. A recent study has shown that n-3 FA supplementation can decrease the frequency of AF in this setting\textsuperscript{9}. It is interesting to note that n-3 FAs have been noted to modulate neutrophil and mast cell activity\textsuperscript{10}, both of which are associated with tissue injury and the occurrence of post-cardiac surgery atrial fibrillation\textsuperscript{11}. As shown clearly by Leaf and colleagues, fish oils have direct, acute effects on ion channels that could contribute to this antiarrhythmic effect. However, epidemiologic (eg., GISSI) and dietary\textsuperscript{12} studies showing a clinical benefit of fish oils typically focus primarily on longer time frames. Attenuation of systemic inflammation and a subsequent
reduction in electrical and structural remodeling due to inflammatory mechanisms is also compatible with these observations. Therapies that decrease systemic inflammation may result in less cardiac inflammatory cell infiltration, oxidant production (by myeloperoxidase and other enzymes), and cardiac myocyte injury resulting from activated inflammatory cells.

Is the role of inflammation in arrhythmia generalizable beyond the post-surgical patient to the broader population of patients suffering from AF (and other arrhythmias)? Inflammatory cell infiltration and or subsequent interstitial fibrosis was characteristic of tissue injury present in atrial biopsies from patients suffering from lone AF\textsuperscript{13} – that is, AF in the absence of coronary disease or other cardiovascular abnormalities. In addition to the impact on structural remodeling, recent studies suggest that anti-inflammatory actions of n-3 FAs may also have important electrophysiologic consequences. The recent identification of resolvin E1 as a ligand for the ChemR23 receptor\textsuperscript{14} suggests that this lipid product of EPA can suppress activation of NF-kB and synthesis of cytokines and chemokines that may facilitate migration of inflammatory cells into stressed (fibrillating, failing) tissues\textsuperscript{15}. Finally, systemic inflammation also promotes the production of thromboxane A2 and prostaglandins (TxA2 and PGF2\textsubscript{α}). Recent studies using receptor knockout mice demonstrate that both of these compounds contribute to atrial tachycardias in mice treated with LPS\textsuperscript{16}. Similar tachycardias were produced in response to an exogenous mixture of TNF-\textalpha and interferons, and production could be blocked with indomethacin. Both of these prostanoids are arachidonic acid (n-6 FA) metabolites. It seems likely, therefore, that n-3 FAs, by suppressing production of these metabolites, will contribute to the attenuation of both the ectopic triggers and the structural remodeling that promote arrhythmias in the setting of a systemic inflammatory state.

### 3. Current challenges and the most important issues for future Research

Important challenges include:
1. Evaluating the relative impact of n-3 FAs on ectopic triggers versus tissue substrate for arrhythmia. What endpoints should be used to evaluate the efficacy of novel anti-arrhythmic therapies? Should the focus be on inflammatory markers, myocyte apoptosis, tissue fibrosis, ion channel activity, arrhythmia inducibility, or arrhythmia duration? Or all of the above?
2. Is there an optimum time for n-3 FA treatment? In other words, can it be “too late” for this approach to be useful? If the primary effect is the prevention of degenerative changes, is there no point in using this therapy on scarred, fibrotic hearts?
3. In the longer term, can the efficacy of relatively large doses of n-3 FAs be achieved with smaller doses of more specific anti-inflammatory lipids (eg., resolvins and related compounds?).

### 4. Areas of overlap with other workshop topic areas

1. Epidemiology of arrhythmia and systemic inflammation: Dr. Christine Albert
2. Impact of n-3 FAs on systemic inflammatory markers: Drs. Balk and Chung
3. Cell signaling involved in TxA2 and PGF2\textsubscript{α} mediated tachycardia: Drs. Anderson, Giles
5. Translating the topic into understanding effects of n3-fatty acids on arrhythmogenesis

Three feasible goals for evaluating the hypothesis that anti-inflammatory effects are important for the therapeutic efficacy of n-3 FAs include: 1) to determine whether n-3 FA treatment can modulate the inflammatory response, tissue injury, fibroblast proliferation and arrhythmia inducibility in well defined experimental animal arrhythmia systems (for example, sterile pericarditis); 2) to evaluate whether a pharmacologic reduction of systemic inflammation (with n-3 FAs or other agents) can reverse changes in the extracellular matrix and/or distribution of gap junctions that can promote reentry; and 3) to define the nature of the interaction (competitive?) between n-3 and n-6 FAs as mediators of ectopic activity, the role of n-3 derived metabolites (eg., resolvin E1, etc.) in modulating this activity, and the signaling pathways and ion channels involved in mediating this response.

6. Citations


C-reactive protein and is associated with postoperative arrhythmia. *Circ.* 1997;96:3542-3548.


Anti-inflammatory Mechanisms of the anti-arrhythmic effects of n-3 fatty acids

August 29, 2005

David R. Van Wagoner, Ph.D.
Department of Cardiovascular Medicine
Cleveland Clinic Foundation

Atrial Fibrillation (AF)

- Incidence is strongly age-related (>10% over 80 yrs old), suggesting an important role for degenerative changes in creating a substrate for AF
- Is an independent risk factor for stroke (5-7x) and mortality (2x)
- Ion channel-blocking antiarrhythmic drugs are ineffective (<50% SR @ 1 year)
- Surgical, ablative interventions are more effective, but traumatic and expensive
- More effective pharmacologic treatments are urgently needed

Framingham analysis

Men and women have a 1 in 4 lifetime risk of developing AF

Lloyd-Jones et al., Circ. 110:1042-6, 2004

Numerous associations between cardiovascular events and a "systemic inflammatory state"

- Ridker and colleagues have shown the value of baseline CRP assays in predicting future cardiovascular events including MI and stroke. (NEJM 336:973, 1997)
- Inflammation has been tightly linked to the atherosclerotic process
- There are relevant parallels in AF

Questions...

- Can novel, anti-inflammatory interventions be identified that can prevent arrhythmias, and/or to delay the progression from paroxysmal to persistent forms?
- Do n-3 fatty acids have anti-inflammatory properties compatible with the above goal?

Mechanisms of AF

Summary of EP changes in permanent human AF

Van Wagoner and Nerbonne, J. Mol. Cell Cardiol. 32:1101-1117, 2000

Lipids modulate the inflammatory response...


Acute changes: Inflammatory cytokines and CRP levels rise and fall following cardiac surgery


Sterile pericarditis model

• Developed by Dr. A.L. Waldo in 1986
• Pericardium is opened via a right thoracotomy. Wires are placed, and the atrial surfaces are sprinkled with talc, then a layer of gauze, all under sterile conditions.
• Atrial fibrillation and flutter can reliably be induced with extrastimuli on POD2-4

Parameter | Sample Point | Arrhythmia (n=7) | No Arrhythmia (n=12) | P | C4b/c, nmol/L | Baseline | 54 (35-60) | 15 (11-36) | 0.014 | After protamine | 88 (63-116) | 50 (26-59) | 0.013 | Day 2 | 130 (34-186) | 39 (25-51) | 0.049 | CRP, mg/L | Baseline | 0.23 (0.15-0.47) | 0.33 (0.15-0.65) | NS | After protamine | 0.12 (0.09-0.24) | 0.15 (0.11-0.33) | 65 (50-75) | Day 2 | 51 (42-76) | 21 (8.7-34) | 0.028 | C4d-CRP, pmol/L | Baseline | 2.0 (1.1-4.9) | 0.7 (0.3-3.0) | NS | After protamine | 85 (43-140) | 21 (8.7-34) | 0.028 | Day 2 | 800 (611-1183) | 525 (208-600) | 0.904

Conclusions: Cardiac surgery with CPB causes a biphasic complement activation. The first phase occurs during CPB and results from the interaction of blood with the extracorporeal circuit. The second phase, which occurs during the first 5 days after surgery, involves CRP, is related to baseline CRP levels, and is associated with clinical symptoms, such as arrhythmia.

CRP changes in canine pericarditis parallel post-op changes

Pericarditis stimulates inflammatory cell infiltration; prednisone can attenuate this...

Steroids modulate CRP levels and arrhythmia inducibility

Atorvastatin attenuates tissue injury in the canine SPC model

Atorvastatin reduced CRP levels and decreased AF duration (POD2)

And the impact of n-3 FAs?
N-3 FA’s for post-op AF

- Two gelatin capsules containing 850 to 882 mg eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as ethyl esters in the average ratio of EPA/DHA 1:2
- started immediately after randomization and continued for at least five days before surgery
- PUFAs in the immediate postoperative
- period (24 to 36 h) were given, if needed, through a nasogastric tube. Treatment with PUFAs continued until hospital discharge.
- Compliance, monitored by pill count, was 98%.

n-3 FA supplements decreased the incidence of PO-AF

L. Calo et al., JACC 45:1723-1728, 2005

Is post-operative AF reflective of “generic,” age-related AF?

Evidence of inflammation in AF

- Frustaci evaluated atrial septal biopsies from 12 pts w/ lone pAF
- All biopsies showed structural abnormalities (fibrosis, hypertrophy)
- 8 had lympho-mononuclear infiltrates with necrosis of the adjacent myocytes
- Results were compatible with myocarditis in 66% of pts, with significant fibrosis in the remainder

A. Frustaci et al., Circulation. 1997;96:1180-1184

CRP is linked with arrhythmia persistence

R. Aviles et al., Circulation. 2003;108:3006

CRP as a risk for future AF

R. Aviles et al., Circulation. 2003;108:3006

What do persistently elevated CRP levels reflect???


CRP binds to apoptotic cells: may act as an opsonin

Aime-Sempe C et al., JACC 34:1577 1999

Evidence of apoptosis in AF tissues


Observations on the response to injury / infection...

- War, even in self-defense, “is not without unwanted side effects...”
- namely, inflammation, tissue injury, and disruption of the innate immune response of professional phagocytic cells. A constant feature of acute inflammation is that PMN arrive at the scene first and mononuclear cells arrive next
Lipids modulate the PMN inflammatory response...

Host Defense
Trauma, Tissue Injury

Acute Inflammation

Chronic Inflammation

Pro-Inflammatory Lipid Mediators

Pro-Inflammatory Lipid Mediators

Neutrophils

Resolution

Pro-Resolution Programs

New Terrain

Protective Lipid Mediators

Lipids

Resolvins

Docosahexaenoic Acid

Arlnine Resolved Lipid Mediators


Inflammatory infiltration leads to atrial fibrosis

10x

20x

103-043

And the impact of n-3 FAs?

Especially DHA, can be metabolized into anti-inflammatory compounds (resolvins) that attenuate neutrophil activation and migration.

Production of resolvins is stimulated by ASA, but not other NSAIDs.

May modulate the extent of tissue injury and fibrosis due to activation of neutrophils and macrophages.

N-3 fatty acids...

CRP levels predict extent of scar, and failure of PV ablation.

Once fibrosis is established, atrial electrical remodeling no longer dominates arrhythmia persistence.

Step 1: Proof of principle

Clinical records

Relationship between C-reactive protein concentrations during glucocorticoid therapy and recurrent atrial fibrillation

John Dernellis*, Maria Paneretou
Department of Cardiology, AHEPA Hospital, 7 Kifissias Avenue, 546 12 Thessaloniki, Greece


Significant Limitations

- Concomitant use of propafenone in all patients would limit use in patients with structural heart disease
- Steroids have serious side effects, with respect to immune function, glucose control, etc.

However, other treatment approaches may also be effective...

Glucocorticoid treatment significantly enhanced freedom from recurrent and persistent AF


Ectopic activity in the PVs frequently triggers AF

M. Haissaguerre et al., NEJM 339:659-666

And the impact of n-3 FAs?

???
Inflammatory tachycardias may be due to TXA2 and PGF2α receptor activation...


**Inflammatory tachycardias**
- Are due to n-6 derived mediators in mice (TXA2 and PGF2α)
- Are PV foci in patients due to systemic inflammation: 1) always, 2) sometimes, or 3) never?
- Do n-3 fatty acids modulate the activity of these ectopic foci?

**Summary**
- Ion channel activity is altered by both transcriptional and post-transcriptional mechanisms in AF
- In patients with persistent AF, CRP levels are more elevated than patients with paroxysmal AF or than in control patients.
- In the context of AF, atrial inflammation is likely to reflect ongoing apoptosis and fibroblast proliferation, resulting in structural remodeling that increases arrhythmia persistence

**And the impact of n-3 FAs?**

???

**Clinical Implications (1)**
- AF persistence depends both on electrical remodeling (channelopathy) and structural remodeling
- In the presence of significant structural remodeling, electrical remodeling is not required for arrhythmia persistence
- Ion channel blocking drugs are likely to be less effective in this context

**Clinical Implications (2)**
- Therapies that target or prevent the development of atrial fibrosis (via the RAAS, inflammatory pathways or other mechanisms) may be more successful than conventional, ion-channel blocking antiarrhythmic drugs
- Early interventions are MUCH more likely to be successful than late
A rational target for further study: N-3 fatty acids!

Thanks to:

CCF Cardiology
Michelle Lamorgese
Laurne Castel
Mina Chung
Mary Ruehr

CCF CT Surgery
Michael Banbury
Delos Cosgrove
Marc Gillinov
Bruce Lytle
Patrick McCarthy
Nicholas Smedira

Marie-Luise Brennan
Albert L. Waldo, (Case Western Reserve University)

Ohio State University
John Bauer
Cynthia Carnes
Robert L. Hamlin

National Institutes of Health
HL-57262, HL-65412, HL38408
1. Topic and Author

Potassium Channel Targeting to Plasma Membrane Lipid Microdomains: Possible n-3 Fatty Acid Effects
Jeffrey R. Martens, Ph.D.

2. Where we stand in 2005.

Voltage-gated K⁺ (Kv) channels are an important determinant of cellular excitability and key components of multiple signal transduction pathways. In the cardiovascular system, Kv channels contribute to the electrical and contractile properties of the heart and vascular smooth muscle by regulating cardiac action potential duration and controlling arterial tone, respectively. Kv channels are polytopic proteins embedded in the plasma membrane with a functional tetramer containing 24 transmembrane domains and multiple surfaces for interaction with surrounding lipids. Therefore, it is no surprise that ion channels, proteins designed to overcome the impermeability of the surface membrane, may be functionally dependent on the constituent lipids of the membrane itself. There is increasing interest in the potential role for cellular lipids in the regulation of channel localization. This is the result of a revised view of membrane organization in which the traditional fluid mosaic model has been updated to reflect a developing appreciation of membrane lipid heterogeneity. The existence of membrane microdomains, particularly those referred to as lipid rafts, has motivated investigators to examine the role of protein-lipid interactions in ion channel localization and function more closely. Lipid rafts are specialized membrane microdomains that are rich in sphingolipids and cholesterol. These rafts have been implicated in the organization of many membrane-associated signaling pathways and are currently the focus of intense interest in the scientific community. The targeting of ion channels to sphingolipid- and cholesterol-rich membrane microdomains has emerged as a novel mechanism of ion channel localization. Biochemical and functional evidence indicate that Kv channels, as well as other important cardiovascular channels, localize to lipid raft microdomains on the cell surface. Perturbation of raft lipid composition often leads to dramatic alterations in channel function. Recently, it has been demonstrated that certain fatty acids, in particular n-3 polyunsaturated fatty acids (PUFA), can remodel raft microdomains. Together, these emerging data indicate that protein-lipid interactions should be considered as a new mechanism of ion channel localization and compartmentation that might permit the modulation of channel properties via alteration in membrane lipids either by disease, diet, or the clinical use of lipid lowering drugs.
3. Current challenges and the most important issues for future Research

Fatty acid-regulation of Kv channels is quite the quandary and the physiological relevance of their action—cardioprotective and arrhythmogenic effects, for example—is complex and has not been fully characterized. Most of the published work on this topic is phenomenological. Cis-polyunsaturated fatty acids applied extracellularly seem to have the greatest effect, be it enhancing or inhibitory, but whether the channels have binding sites or the action is more of a simple electrostatic interaction is unknown. The potential role of polyunsaturated fatty acids in selectively modulating channel function by perturbation of lipid microdomain composition and organization remains unanswered.

In addition, important questions regarding channel-raft interactions remain. An elucidation of mechanisms for channel-raft and channel-caveolae association is important for understanding protein-lipid interactions but may also lead to an understanding of the functional significance of microdomain localization. Obviously, additional work is needed is to understand how lipid raft-channel association is integrated into the broader context of normal cellular signaling and the pathogenesis of disease.

4. Areas of overlap with other workshop topic areas

Lipid microdomains have emerged as important signaling centers for compartmentation of signaling transduction machinery and an interface for protein-lipid interactions. In addition to the regulation of ion channel activity, lipid rafts are proposed to play important roles in a number of areas discussed in this workshop including excitation-contraction coupling, immune response, and recently the magnitude and specificity of calcium/calmodulin-dependent protein Kinase II phosphorylation of substrates.

5. Translating the topic into understanding effects of n3-fatty acids on arrhythmogenesis

As with all excitable tissues, Kv channels play an essential role in the complex electrical responses of the cardiovascular system. These channels, which are targets of several antiarrhythmic drugs, open and close in response to a change in membrane voltage and are responsible for establishing the resting membrane potential and determining repolarization. One example, Kv1.5—a prominent cardiovascular K⁺ channel expressed in the atrium, ventricle, and SA-node, mediates the ultrarapid potassium current (I_{Kur}) that augments late cardiac action potential repolarization and therefore regulates action potential duration. In the atria, a role for Kv1.5 in both normal and pathological conditions, such as atrial fibrillation, is established.

Another example includes HCN channels, which encode for the pacemaker (I_{f}) current in the sinoatrial node. Both Kv1.5 and HCN channels have been localized to lipid rafts and disruption of these microdomains alters current properties. Importantly, n-3 PUFAs alter the protein and lipid composition of lipid rafts/caveolae. This raises the possibility of regulating Kv channel function, and therefore cardiac arrhythmogenesis, based on channel protein/lipid interactions within rafts/caveolae via the dietary intake of polyunsaturated fats.
6. Citations

Potassium Channel Targeting to Plasma Membrane Lipid Microdomains: Possible n-3 Fatty Acid Effects

Jeffrey R. Martens

Important Role of Kv Channels in Heart

<table>
<thead>
<tr>
<th>AP Phase</th>
<th>Ionic Current</th>
<th>Channel Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$I_{Na}$ (in)</td>
<td>SCN5A</td>
</tr>
<tr>
<td>1</td>
<td>$I_{Na}$ (out)</td>
<td>Kv4.3</td>
</tr>
<tr>
<td>2</td>
<td>$I_{Ca}$ (in)</td>
<td>a1C</td>
</tr>
<tr>
<td>2</td>
<td>$I_{Na/Ca}$ (in/out)</td>
<td>NCX1</td>
</tr>
<tr>
<td>3</td>
<td>$I_{Kur}$ (out)</td>
<td>Kv1.5</td>
</tr>
<tr>
<td>3</td>
<td>$I_{Kv}$ (out)</td>
<td>HERG</td>
</tr>
<tr>
<td>4</td>
<td>$I_{Ks}$ (out)</td>
<td>KvLQT1</td>
</tr>
<tr>
<td>4</td>
<td>$I_{K1}$ (out)</td>
<td>Kv2.1</td>
</tr>
</tbody>
</table>

Important Role of Kv Channels in Vascular Smooth Muscle

Much is Known about Kv Channel Structure/Function

Model for the Organization of Lipid Rafts in the Plasma Membrane

Partial List of Proteins Localized to Lipid Rafts

<table>
<thead>
<tr>
<th>Class of protein</th>
<th>Name</th>
<th>Biochemical localization</th>
<th>Morphological localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosphingolipid-anchored (GPI)</td>
<td>Folate receptor</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Membrane receptor</td>
<td>PDGF</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Membrane receptor</td>
<td>EGF</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Membrane receptor</td>
<td>β-adrenergic</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Membrane receptor</td>
<td>Bradykinin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Membrane receptor</td>
<td>Endothelin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Membrane receptor</td>
<td>Heterotrimeric Gα, Gβ</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Membrane receptor</td>
<td>Src, Fyn, Hck</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Membrane receptor</td>
<td>E-Nos</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Membrane receptor</td>
<td>PKC</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Membrane transporter</td>
<td>IP3 receptor</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Membrane transporter</td>
<td>Ca2+ATPase</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

1. Lipid bilayer surrounding membrane
2. GPI-anchored protein of McArdle cells, including yeast
3. Involved in caveolar trafficking in monocytes
4. Cholesterol depletion leads to lipid raft formation

K +

Importance of Kv Channels in Blood Vessel Smooth Muscle:

Adapted from: Rasmusson et al., Circ Res. 82:739-750 (1998)

Adapted from Long et al., Science, Vol 309, Issue 5736, 897-903, 2005

Phylogenetic tree

Adapted from: Hooper N. M., Molecular Membrane Biology 16:145-156 (1999)
Diseases for which Rafts and Raft Proteins are Targets

Adapted from Simons & Ehehalt; J.Clin. Invest. 2002, 110 (5) 597-603

n-3 Polyunsaturated Fatty Acids Perturb Lipid Rafts

1. alter the lipid composition of rafts
2. change membrane properties
3. displace proteins from lipid microdomains
4. alter protein acylation

Caveolin: The Mechanism of Kv1.5 Raft Association

Adapted from: Martens et al., Trends Pharm. Sci. 2004; 25(1):16-21

Channel Association with Lipid Rafts/Caveolae

What is the role of modulating dietary lipid composition, n-3 PUFA, in selectively regulating raft-associated ion channel function?
Kv channels play an essential role in the complex electrical responses of the cardiovascular system. Biochemical and functional evidence indicate that Kv channels, as well as other important cardiovascular channels, localize to lipid raft microdomains on the cell surface. Perturbation of raft lipid composition often leads to dramatic alterations in channel function.  n-3 polyunsaturated fatty acids (PUFA) can remodel raft microdomains.

We propose that the dietary intake of polyunsaturated fats may regulate Kv channel function, and therefore cardiac arrhythmogenesis, based on channel protein/lipid interactions within rafts/caveolae. The mechanism of this effect may include a change in membrane properties, displacement of channel proteins from lipid microdomains, and/or alteration of protein acylation.

Summary

- Kv channels play an essential role in the complex electrical responses of the cardiovascular system.
- Biochemical and functional evidence indicate that Kv channels, as well as other important cardiovascular channels, localize to lipid raft microdomains on the cell surface.
- Perturbation of raft lipid composition often leads to dramatic alterations in channel function.
- n-3 polyunsaturated fatty acids (PUFA) can remodel raft microdomains.

We propose that the dietary intake of polyunsaturated fats may regulate Kv channel function, and therefore cardiac arrhythmogenesis, based on channel protein/lipid interactions within rafts/caveolae. The mechanism of this effect may include a change in membrane properties, displacement of channel proteins from lipid microdomains, and/or alteration of protein acylation.

Michael Mayer, Ph.D.
Assistant Professor
Biomedical Engineering and Chemical Engineering
University of Michigan

Martens’ Laboratory

OHSU
James Brady
Karyn Foster
Barbara Leighton
Kimberlee Stafford

University of Michigan
Saif Jackson
Qiuju Li
RaShonda Flint
Dave Dudek
Lian Zhang
Omega-3 Fatty Acids and their Role in Cardiac Arrhythmogenesis Workshop
Embassy Suites Hotel, Washington D.C.
August 29-30, 2005

1. Topic and Author

The acute effects of omega-3 fatty acids in large animal models.
George E. Billman, Ph.D., F.A.H.A.

2. Where we stand in 2005.

Sudden cardiac death (defined as unexpected death from cardiac causes that occur within 1 hour after the onset of symptoms) remains the leading cause of death in industrialized countries, accounting for between 300,000 and 500,000 death each year in the United States (1). Holter monitoring reveals that these sudden deaths most frequently (up to 93 percent) resulted from ventricular tachyarrhythmias (2-4). Yet, despite the enormity of this problem, the development of safe and effective antiarrhythmic agents remains elusive. In fact, several initially promising antiarrhythmic drugs have actually been shown to increase, rather than decrease, the risk for arrhythmic death in patients recovering from myocardial infarction (5-6). Furthermore, even the best currently available therapies (i.e., amiodarone, 7, or beta-adrenergic receptor antagonists, 8-11) reduce, rather than completely eliminate, sudden death in high risk patients and these agents also frequently exhibit untoward side effects. Therefore, non-pharmacological interventions should be examined to determine whether they might provide a better therapeutic option.

There is an increasing body of evidence that suggests that diets rich in omega-3 polyunsaturated fatty acids can prevent ischemically-induced ventricular fibrillation in animals (12) as well as reduce the incidence of sudden death in patients recovering from myocardial infarction (13). Recently, we investigated the effects of the acute intravenous administration of omega-3 fatty acid using a canine model of ventricular fibrillation (14). Briefly, ventricular fibrillation (VF) was induced by a 2-minute occlusion of the left circumflex coronary artery during the last minute of submaximal exercise (running on a treadmill) in dogs with healed anterior wall myocardial infarctions. This exercise plus ischemia test induced ventricular fibrillation in the 27 of the 44 dogs tested. On a subsequent day, the exercise plus ischemia test was repeated in susceptible animals (i.e. had VF) after one of the following treatments. First, the effects of an emulsion of concentrated fish oil (1g to 10g, 25% docosahexaenoic acid, DHA and 34% eicosapentaenoic acid, EPA) infused over 60 minutes (1.5 ml/min) prior to the onset of exercise were examined (15,16). The fish oil infusion elicited significant reductions in heart rate (both at rest and during exercise), QTc interval, and left ventricular systolic pressure while increasing P-R interval. This intervention prevented VF in 10 of 13 dogs tested. In contrast to the fish oil emulsion, the pure omega-3 fatty acids did not alter resting (i.e., pre-exercise) heart rate, PR...
interval or QTc interval. The results indicate that intravenous administration of either fish oil or purified omega-3 fatty acids can prevent ischemia-induced ventricular fibrillation. The mechanism responsible for this protection remains to be determined in intact preparations. However, these compounds have been found to have potent effects on sodium and calcium channels (see 18,19). It is likely that the antiarrhythmic action of the omega-3 fatty acids results from actions on these ion channels (sodium channel inactivation and inhibition of the L-type calcium current, preventing calcium overload associated with myocardial ischemia.).

3. Current challenges and the most important issues for future Research

As noted above, the mechanism mediating the antiarrhythmic actions of omega-3 fatty acids remains to be determined in intact preparations. It remains to be determined what are the electrophysiological effects of the omega-3 fatty acids in the intact heart at rest and during ischemia. What are the effects of these agents on reentrant and non-reentrant arrhythmias? What are the effects on ventricular activation and repolarization (EP mapping studies)? Given the possible actions on the L-type calcium current, what are the effects of these agents on cardiac mechanical properties? Would these agents worsen mechanical impairment induced by ischemia? Could they be detrimental in patients with poor cardiac function? There are several important unanswered questions concerning the effective dose for the antiarrhythmic protection provided by omega-3 fatty acids as follows: What is the minimally effective dose? What is the half-life, duration of action of these substances? What is minimal time before dietary omega-fatty acids become effective?

4. Areas of overlap with other workshop topic areas

Areas of possible overlap include:
1. Role of calcium-calmodulin interactions in arrhythmogenesis: possible sites of n-3 fatty acid modulation.
2. Potassium channel targeting of plasma membrane lipid microdomains: possible n-3 fatty acid effects.
3. Possible sites of n-3 fatty acid actions on electromechanical activity.

5. Translating the topic into understanding effects of n3-fatty acids on arrhythmogenesis

Omega-3 fatty acids have significant antiarrhythmic effects in a canine model of sudden death. In vitro studies provide strong evidence that these lipids alter a number of important cardiac ion channels, particularly sodium and calcium channels. It is likely that the inhibition of these ionic currents is ultimately responsible for the antiarrhythmic actions of omega-3 fatty acids. Additional studies are required to determine the precise electrophysiological events that are responsible for the cardioprotective effects of the omega-3 fatty acids.
6. Citations


19. Leaf A, Kang JX, Xiao Y-F and Billman GE. The clinical prevention of sudden cardiac
The Acute Effects of Omega-3 Fatty Acids in Large Animal Models

George E. Billman, Ph.D., F.A.H.A.
Department of Physiology and Cell Biology
The Ohio State University

Prehistoric Sudden Cardiac Death

Sudden Death Background Information

- Sudden death is the leading cause of death in industrial countries. In the United States, 300,000 to 500,000 die suddenly each year. (Zheng et al., *Circulation* 104: 2158-2163, 2001; Abildstrom et al., *Cardiac Electrophysiol Rev* 3: 177-179, 1999)
- Only minority of these patients had a known history of heart disease yet up to 90% were shown to have coronary artery disease post mortem
- “Only about 1% of the victims of cardiac arrest are resuscitated and survive to leave the hospital.” (Bigger, *Cardiac Electrophysiol Rev* 1/2: 198-204, 1997)

Results of the CAST Study

- Survival among 1455 patients randomly assigned to receive encainide or flecainide or matching placebo.
  - Cause of Death was arrhythmia or cardiac arrest.

Results of the GISSI-Prevenzione Study


Cardiac Instrumentation

- Diagram of cardiac instrumentation showing structures such as atria, ventricles, and coronary arteries.
Exercise Plus Ischemia test

Exercise Level

4.8 kph
6.4 kph

Occlusion

Exercise + Ischemia Test

Myocardial Infarction
(n = 762, 212 died with 72 hr)

Susceptible (had VF, n = 300, 59.2%)
Defib* (n = 255)
No Defib (n = 45, 15.0%)

Defib* Only 5.9% (15 of 255) did not have VF on a 2nd test

Resistant (no VF, n = 207)

Effect of Submaximal Exercise on Heart Rate Variability

Effect of Coronary Arterial Occlusion of Heart Rate Variability

Wavelet Analysis

Susceptible
Resistant
**Myocardial Infarction**

(n = 70, 26 died within 72 hr)

**Exercise + Ischemia Test**

(n = 44, occluder failure n = 3)

**Susceptible**

(had VF, n = 27, 65.9%)

**Resistant**

(no VF, n = 14)

**Fish Oil**

(n = 47)

**Soy Oil**

(n = 7)

**EPA**

(n = 7)

**DHA**

(n = 8)

Fish Oil

EPA

DHA

Soy Oil

(Billman et al., Lipids 32: 1161-1168, 1997)

**Effect of Fish Oil on Cardiovascular Variables**

(Billman et al., Lipids 32:1161-1168, 1997)

(Billman et al., Lipids 32:1161-1168, 1997)

Effect of Fish Oil on Heart Rate Variability

(Billman et al., Lipids 32:1161-1168, 1997)

Effect of Fish Oil on Susceptibility to Sudden Death

(Billman et al., Lipids 32:1161-1168, 1997)
Effect of Fish Oil on Susceptibility to VF

Effect of Omega-3 Fatty Acids on Sudden Death

Effect of DHA on Calcium Transients and Myocyte Shortening
Effect of EPA (1.5 µM) on L-type Calcium Current in Adult Rat Cardiomyocytes

Effect of EPA (5 µM) on Resting and Inactivated Human Cardiac Na⁺ Channels

Effect of EPA (10 µM) on Action Potential Threshold Current

Conclusions

- Dietary Omega-3 Fatty Acids Reduce the Incidence of Sudden Death in Patients and Prevent Ventricular Fibrillation Induced by Myocardial Ischemia in Animal Models
- Acute Intravenous Administration of Emulsions of Omega-3 Fatty Acids Protect Against Ventricular Fibrillation Induced by Myocardial Ischemia in Conscious Canine Model of Sudden Cardiac Death
- The Antiarrhythmic Effects of Omega-3 Fatty Acids Most Likely Result from Inhibition of Ion Channels, Particularly Calcium and Sodium Channels
Acknowledgements

The Ohio State University:
• Monica Kulkielka

Harvard University:
• Alexander Leaf
• Jing X. Kang
• Yong-Fu Xiao
1. Topic and Author

Possible Sites of n-3 Fatty Acid Actions on Cardiac Electromechanical Activity
Wayne R. Giles, Ph.D.

2. Where we stand in 2005.

There is now substantial evidence that in the mammalian heart omega-3 fatty acids (in micromolar concentrations) can alter cardiac excitability and reduce the incidence of arrhythmias and sudden death. Although the cellular and molecular mechanisms for these effects have not been elucidated fully, previous myocyte electrophysiology and heterologous expression experiments clearly demonstrated a significant inhibitory effect of these agents on the human cardiac sodium current, I_{Na}. Perhaps the most relevant experimental work, involving co-expression of the alpha and selected beta sub-units for I_{Na} demonstrated a potent inhibition, with an indication of voltage dependent block and altered kinetics of inactivation and reactivation.

Animal studies and clinical trials have also demonstrated protective effects of fish oil diets and/or intravenous administration of omega-3 fatty acids when measured as incidence of sudden death in humans; or of ischemia-induced rhythm disturbances in animal models. Many of these effects have been interpreted in terms of the well-established ability of omega-3 fatty acids to inhibit sodium current.

Recent studies, however, have also demonstrated significant inhibitory effects of these same agents (EPA, DHA) on the alpha sub-unit of a potassium channel (KV 4.3), which is largely responsible for the calcium-independent transient outward current, Ito, in human heart. These effects also have been reported to occur at or near the concentrations which correspond to plasma levels in humans and larger animals. This inhibitory effect of FFA also involves a voltage-dependent mechanism with altered steady-state inactivation and changes kinetics of reactivation. These effects on K+ currents are important. It is known that Ito contributes significantly to the "shaping" of early repolarization, and thereby alters the calcium transient and excitation-contraction coupling. A complex interaction between I_{Na} and Ito is the basis for a major working hypothesis concerning some of the rhythm disturbances referred to as "the Brugada Syndrome."

3. Current challenges and the most important issues for future Research

Few single myocyte electrophysiological studies have been done under conditions which are designed to mimic ischemia or reperfusion following ischemia. However, it is known, that in the setting of ischemia or in some models of hypoxia, I_{Na} in the ventricle is altered such that the
peak current is decreased, and a non-inactivating or persistent inward current emerges. This same pattern of electrophysiological change is seen in the setting of increased free radicals (specifically H$_2$O$_2$ levels). It will be of interest, therefore, to explore the actions of EPA and DHA on this potential target in models of ischemia and reperfusion.

Although there is no convincing evidence that either EPA or DHA directly or significantly alters intercellular conductance in mammalian ventricle, it is known that both oleic acid and stearic acid can alter syncytial function. As a result, both basic science and clinical studies will require careful monitoring of the entire lipid profile of the animal model or patient in order to ascertain the single most important electrophysiological change which triggers or strongly modulates the altered electrical or mechanical activity.

Recent mathematical models of human atrium and ventricle may be helpful in integrating these somewhat diverse experimental results. To the extent that this is possible, more insight can be gained from both single cell experiments and in vivo models of spontaneous or inducible arrhythmias.
Possible sites of n-3 Fatty Acid Actions on Cardiac Electromechanical Activity

Ion Channel Targets

W. Giles
Dept. of Bioengineering and Medicine
UCSD
wgiles@bioeng.ucsd.edu

I. Background
- fatty acid synthesis/catabolism
- plasma levels

II. Methods
- ECG
- Patch Clamp
- V-scn Dyes
- Math Models

III. Ion channel Targets
- $I_{\text{Na}}$
- $I_{\text{K}}$
- $I_{\text{Ca}}$

IV. Simulations of Human Ventricle Model

V. Summary

Ventricular Arrhythmias

$I_{\text{Na}}$

Simulations of Human Ventricle Model

$I_{\text{Na}}$

Simulations of Human Ventricle Model

Simulations of Human Ventricle Model
Inotropic effect of reducing external [Ca\(^{2+}\)]

Mouse Raw Data Traces

Mouse Raw Data Traces

Prolongation of rat ventricular action potential by H\(_2\)O\(_2\)
INa block by 80%, beginning at $t = 999$ ms

Nygren and Giles, 2000

Gussak and Antzelevitch, 2003

Antzelevitch, 2005

Acknowledgements

- R. Clark
- C. Kondo
- M. Fink

Table 11.3 Ion currents, their subunits, encoding genes and chromosome location.

<table>
<thead>
<tr>
<th>Current</th>
<th>Gene</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>INa</td>
<td>SCN5A</td>
<td>3p21</td>
</tr>
<tr>
<td>α-subunit</td>
<td>β1 (SCN5B)</td>
<td>19q13.1-q13.2</td>
</tr>
<tr>
<td>β-subunit</td>
<td>β2 (SCN5B)</td>
<td>11q23</td>
</tr>
<tr>
<td>ICaL</td>
<td>α1C (GKAC1LA1)</td>
<td>12pter-p13.2</td>
</tr>
<tr>
<td>α-subunit</td>
<td>β1 (GKAC1LA1)</td>
<td>17q21-q22</td>
</tr>
<tr>
<td>β-subunit</td>
<td>β2 (GKAC1LA1)</td>
<td>10q12</td>
</tr>
<tr>
<td>ICaR</td>
<td>α1C (GKAC1LA2)</td>
<td>7q21-22</td>
</tr>
<tr>
<td>INa</td>
<td>Kv4.3 (KCND3)</td>
<td>1p13.2</td>
</tr>
<tr>
<td>α-subunit</td>
<td>β1 (KCND3)</td>
<td>16q34</td>
</tr>
</tbody>
</table>
Roster

Omega-3 Fatty Acids and their Role in Cardiac Arrhythmogenesis
Workshop: Research Challenges and Opportunities
August 29-30, 2005

Barry London, M.D., Ph.D. (Chair)
University of Pittsburgh Medical Center
Cardiovascular Institute
200 Lothrop Street
Scaife 572
Pittsburgh. PA 15213-2582
E-mail londonb@upmc.edu

Wayne R. Giles, Ph.D.
University of California at San Diego
Department of Bioengineering
9500 Gilman Drive, Dept. 0412
La Jolla, CA 92093-0412
E-mail wgiles@bioeng.ucsd.edu

Mark E. Anderson, M.D.
Vanderbilt University Medical Center
Department of Medicine
2220 Pierce Ave, 383 PRB
Nashville, TN 37232-6300
E-mail mark.anderson@vanderbilt.edu

Alexander Leaf, M.D.
Massachusetts General Hospital
Department of Medicine
55 Fruit Street
Boston, MA 02114-2696
E-mail aleaf@partners.org

Christine Albert, M.D.
Brigham and Women's Hospital
Department of Medicine
900 Commonwealth Ave, E
Boston, MA 02215
E-mail calbert@partners.org

Bill Lands, Ph.D.
6100 Westchester Park Drive
Apartment 1219
College Park, MD 20740-2852
E-mail wemlands@att.net

Ethan Balk, M.D., M.P.H.
Tufts-New England Medical Center
Department of Medicine
Division of Clinical Care Research
750 Washington Street, #63
Boston, MA 02111
E-mail EBalk@tufts-nemc.org

Jeffrey R. Marten, Ph.D.
University of Michigan
Department of Pharmacology
1150 W. Medical Center Drive
1301 MSRB III
Ann Arbor, MI 48109-0632
E-mail martensj@umich.edu

George E. Billman, Ph.D.
Ohio State University
Department of Physiology & Cell Biology
1645 Neil Ave.
304 Hamilton Hall
Columbus, OH 43210-1218
E-mail billman.1@osu.edu

John H. McAnulty, M.D.
Good Samaritan Hospital
1130 NW 22nd Ave
Portland, OR 97210
E-mail imcanult@lhs.org; mcanultj@ohsu.edu

Mei Chung, M.P.H.
Tufts-New England Medical Center
Department of Medicine
Division of Clinical Care Research
750 Washington Street
Boston, MA 02111
E-mail MChung1@tufts-nemc.org

David Van Wagoner, Ph.D.
Cleveland Clinic Foundation
Department of Cardiovascular Medicine
9500 Euclid Ave., FF-10
Cleveland, OH 44195
Tel. (216) 444-0820
E-mail vanwagd@ccf.org
NIH Staff

**David A. Lathrop, Ph.D.**
Leader-- Arrhythmias, Ischemia, and Sudden Cardiac Death Research Group
Heart Research Program
Division of Heart and Vascular Diseases
National Heart, Lung, and Blood Institute
National Institutes of Health
6701 Rockledge Drive, Room 9192
Bethesda, MD 20892-7940
E-mail LathropD@nhlbi.nih.gov

**Rebecca B. Costello, Ph.D.,F.A.C.N.**
Deputy Director
Office of Dietary Supplements
National Institutes of Health
6100 Executive Blvd., Room 3B01, MSC 7517
Bethesda, Maryland 20892-7517
E-mail CostellB@od.nih.gov

**Isabella Liang, Ph.D.**
Health Scientist Administrator
Arrhythmias, Ischemia, and Sudden Cardiac Death Research Group
Heart Research Program
Division of Heart and Vascular Diseases
National Heart, Lung and Blood Institute
National Institutes of Health
6701 Rockledge Drive, Room 9194
Bethesda, MD 20892-7940
E-mail LiangI@mail.nih.gov