What is the Data on Fish Oil for Peripheral Artery Disease?

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UCSF VASCULAR SURGERY SYMPOSIUM
SAN FRANCISCO, CA
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• There are no conflicts of interest

...In fact, I am allergic to fish and seafood...

Goals

• Review the biological effects of n-3 PUFA on vasculature

• Summarize the evidence for the use of n-3 PUFA in cardiovascular disease

• Determine if n-3 PUFA could improve treatment of patients with PAD

Nutritional Patterns

• Nutritional patterns have changed considerably in the last century
  – Industrial revolution ~60s
  – Increase in corn oil
    • Grenon et al., Vasc Med (2012)
• Polyunsaturated fatty acids (PUFAs) ratios altered (n-6:n-3)
  – Hunter-Gatherers: 2:1
  – American Diet: 15:1
A Healthy Diet...
American Heart Association

- Balance calorie intake and physical activity to achieve or maintain a healthy body weight
- Diet rich in vegetables and fruits
- Whole-grain, high-fiber foods
- Minimize intake of beverages and foods with added sugars
- Choose and prepare foods with little or no salt
- Alcohol in moderation
- When you eat food that is prepared outside of the home, follow the AHA diet and lifestyle recommendations.
- Limit intake of saturated fat to <7% of energy, trans fat to <1% of energy and cholesterol to <300 mg/day
- **Consume fish, especially oily fish, at least twice a week**

Rich in n-3 PUFA


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2002 AHA Recommendations:
Fish and fish oil

<table>
<thead>
<tr>
<th>Patient Population</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients without documented coronary heart disease (CHD)</td>
<td>Eat a variety of (preferably fatty) fish at least twice a week. Include oils and foods rich in alpha-linolenic acid (flaxseed, canola and soybean oils; flaxseed and walnuts).</td>
</tr>
<tr>
<td>Patients with documented CHD</td>
<td>Consume about 1 g of EPA+DHA per day, preferably from fatty fish. EPA+DHA in capsule form could be considered in consultation with the physician.</td>
</tr>
<tr>
<td>Patients who need to lower triglycerides</td>
<td>2 to 4 grams of EPA+DHA per day provided as capsules under a physician’s care.</td>
</tr>
</tbody>
</table>

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What are fatty acids?

- **Fatty Acids**
  - Carboxylic acid with a long unbranched chain which is either saturated or unsaturated
- **Unsaturated**
  - Double-bond in the fatty acid chain
  - When double-bonds are formed, hydrogen is eliminated
  - Confers different biological properties

![Fatty Acid Diagram](image)

- Rich in n-3 PUFA

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Essential Fatty Acid Families

**ω-6 Family**

- **Linoleic**
  - C18:2
  - Corn Oil
  - Safflower Oil
  - Sunflower Oil
- **Arachidonic**
  - C20:4
  - Poultry
  - Meats

**ω-3 Family**

- **α-Linolenic**
  - C18:3
  - Flaxseed Oil
  - Canola Oil
  - Soybean Oil
- **Docosahexaenoic (DHA)**
  - C22:6
  - Oily Fish
  - Fish Oil Capsules

More thrombotic and inflammatory metabolites

Less thrombotic and inflammatory metabolites

With permission from Harris WS
Where are they found?

Postulated Effects of n-3 PUFA

- Reduction of serum triglycerides
- Incorporation in the phospholipids of cell membranes, replacing AA with potentially less pro-thrombotic and vasoconstrictive derivatives
- Direct effects on endothelial activation
  - Reduction in production of cytokines (IL-1 and TNF in LPS-stimulated monocytes)
  - Reduction in production of PDGF-A and –B protein and mRNA
  - Reduction of tissue factor by monocytes
  - Increased bioavailability of endothelial nitric oxide
  - Downregulation of gene expression of MCP-1
  - Reduced expression of endothelial adhesion molecules (VCAM-1, E-Selectin, and to less extent ICAM-1)
  - Reduction in VCAM-1 mRNA by Northern blot
  - Reduction in NF-κB system of transcription factors (this controls the coordinated expression of adhesion molecules and of leukocyte-specific chemoattractants upon cytokine stimulation)
- Reduced monocyte cell adhesion to cytokine-activated endothelium
Old tale or the new tale...

**Old tale**
- Resolution of inflammation is a passive process
- No active participation of lipid mediators in resolution process
- Mediators:
  - Decrease cytokines
  - Decrease PGs
  - Decrease oxygen species

**New tale**
- Resolution is an active process
- Lipid mediators are actively involved in the resolution by switching their phenotype
- Mediators:
  - Resolvins
  - Protectins
  - Lipoxins
  - Aspirin-triggered lipoxins
  - Maresins

**Inflammation and Resolution of Inflammation**

Fredman and Serhan, Biochem J 2011

Stables and Gilroy, Prog Lip Res 2011

Chemical Mediators

- Arachidonic acid (AA)
- Eicosapentaenoic acid (EPA)
- Docosahexaenoic acid (DHA)
- Prostaglandins
- Leukotrienes
- Thromboxanes
- Lipoxins
- E-Series Resolvins
- D-Series Resolvins
- Protectins
- Maresins

Fredman and Serhan, Biochem J 2011

Effects of resolution mediators on cell function

- n-3 PUFA are tightly related to inflammation and resolution of inflammation
- Patients with PAD may have a deficit in the resolution of inflammation
Goals

• Review the biological effects of n-3 PUFA on vasculature

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Potential EPA and DHA Effects

• Reduced atherosclerosis
• Antiarrythmic effects
• Improvement in autonomic function
• Decreased platelet aggregation
• Vasodilation
• Decreased blood pressure
• Anti-inflammatory
• Improvement in endothelial function
• Plaque stabilization
• Reduced free fatty acids and triglycerides
• Up-regulation of adiponectin synthesis
• Reduced collagen deposition

Diet and Reinfarction trial (DART)

• First large randomized controlled trial
• Dietary intervention with fish in the secondary prevention of MI.
  – Reduction in fat intake with an increase in the ratio of PUFAs to saturated fat
  – Increase in the fatty fish intake
  – Increase in cereal fibre intake
• 2033 men who had recovered from MI

DART: Reduction in mortality

• The subjects advised to eat fatty fish experienced a 29% reduction in 2-year all-cause mortality compared to those not so advised.
**Gissi Trial**

- Randomized study
- 11,323 patients with recent MI (< 3 months)
- Treated and followed for 3.5 years
  - Omega-3 FAs (1gm/day; EPA/DHA 1:2) (n=2836)
  - Vitamin E (300mg/day) (n=2830)
  - Omega-3 + Vitamin E (n=2830)
  - Controls (n=2828)
- Primary endpoint: composite of death, nonfatal MI and stroke
- Analysis: intention-to-treat analysis.

Gruppo Italiano per lo Studio della Streptochinasi nell’Infarto Premozione Investigatori, Lancet 1999

**Japan EPA Lipid Intervention Study (JELIS- 2007)**

- Prospective, randomized open-label, blinded endpoint evaluation
- 18,645 patients with total cholesterol of 6.5mmol/L or greater
- Randomized to:
  - 1800mg of EPA daily with statin (n=9326)
  - statin only (n=9319)
- 5 year follow-up, intention-to-treat analysis
- Primary endpoints: MCE

Yokoyama et al. (JELIS), Lancet 2007

**Gissi Trial**

- Omega-3 FAs reduced the primary end point by 15% (p=0.023).
- Mortality was significantly lower after 3 months of treatment.
- Sudden death was significantly decreased starting at 4 months.
- Decrease in cardiovascular, cardiac and coronary deaths were observed after 6-8 months of treatment.

Gissi Prevenzione Investigators, Lancet 1999

**JELIS trial: Reduction in MCE**

Yokoyama et al. (JELIS), Lancet 2007
The OMEGA trial

- First randomized study on the effects of highly purified omega-3 acid ethyl ester \textit{in addition to current guideline therapy} 3-14 days after MI
- 1gm/day for 1 year
- Double-blinded, multicenter trial
- N=3,851
- Primary endpoint: SCD
- Secondary endpoints: total mortality and non-fatal events

Rauch et al, Circulation 2010, 122:2152-2159

The OMEGA trial

- Very Underpowered (20%)
- Dose far too low
- Fish consumption increased significantly

Eckel RH, Circulation 2010
Since the pathogenesis of coronary heart disease and peripheral arterial disease (PAD) is closely related, could n-3 PUFA also be effective in the treatment and prevention of PAD?

Goals

- Review the biological effects of n-3 PUFA on vasculature
- Summarize the evidence for the use of n-3 PUFA in cardiovascular disease
- Determine if n-3 PUFA could improve treatment of patients with PAD
Summary of PAD studies

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Design</th>
<th>Patients</th>
<th>Duration</th>
<th>Intervention</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woodcock et al.</td>
<td>1994</td>
<td>Double-blind, randomized, placebo-controlled study</td>
<td>M &amp; W 68</td>
<td>10</td>
<td>3 months</td>
<td>Decreased in blood viscosity, decreased in TG</td>
</tr>
<tr>
<td>Cost et al.</td>
<td>1996</td>
<td>Double-blind, randomized, placebo-controlled study</td>
<td>IC 32</td>
<td>3 months</td>
<td>Walking distance</td>
<td>Decreased in viscosity, improved in lipid profile</td>
</tr>
<tr>
<td>Long et al.</td>
<td>1998</td>
<td>Double-blind, randomized, placebo-controlled study</td>
<td>M &amp; F 120</td>
<td>3 years</td>
<td></td>
<td>No change in lipid profile, decreased in plasma viscosity</td>
</tr>
<tr>
<td>Carvasso et al.</td>
<td>2005</td>
<td>Double-blind, randomized, placebo-controlled study</td>
<td>M 60</td>
<td>12 months</td>
<td>Walking distance</td>
<td>Decreased in TC, increased in HDL</td>
</tr>
<tr>
<td>Mollison et al.</td>
<td>2007</td>
<td>Prospective Study</td>
<td>M 69</td>
<td>12 months</td>
<td>Walking distance</td>
<td>Increase in walking distance, decreased in ABI</td>
</tr>
<tr>
<td>Schimme et al.</td>
<td>2007</td>
<td>Single-blinded, randomized trial</td>
<td>M &amp; F 32</td>
<td>3 months</td>
<td>Walking distance</td>
<td>Increase in walking distance to start pain, decreased in walking distance</td>
</tr>
</tbody>
</table>

Cochrane Review 2007

- 6 studies
- 313 patients with IC
- Omega-3 dietary supplementation vs placebo
- Treatment duration between 4 weeks and 2 years


Polyunsaturated fatty acids and peripheral artery disease

S Marlene Grenon et al., Millie Hughes-Fulford et al., Joseph Rapp et al., and Michael S Conte

Abstract

There is substantial evidence that polyunsaturated fatty acids (PUFAs) such as n-3 and n-4 fatty acids (FAs) play an important role in prevention of atherosclerosis. In vitro and in vivo studies focusing on the interactions between monocytes and endothelial cells have explored the molecular effects of FAs on these interactions. Epidemiological surveys, followed by large, randomized, control trials have demonstrated a reduction in major cardiovascular events with supplementation of n-3 FAs in secondary prevention settings. The evidence of beneficial effects specific to patients with peripheral artery disease (PAD) remains elusive, and is the focus of this review.

PAD Meta-analysis...

- Only advantage appears to be a limited hematological benefits (plasma viscosity)
- No changes in:
  - ABI
  - Walking distance
  - Blood Pressure
  - Triglycerides
- But...
  - Small number of patients
  - Short follow-up
  - May be targeting a disease degree that is more benign hence effects may be more difficult to see
  - Measures of vascular function and inflammation missing
Randomized, double-blinded, placebo-controlled trial

Team:
- PI: SM Grenon
- Co-i: MS Conte, C Owens, J Rapp

Endpoints:
- Primary: Endothelial function
- Secondary: Inflammation

Population:
- N=80, claudicants at the SF VAMC and UCSF

Design of the OMEGA-PAD trial

PAD, nutrition and inflammation

- Inflammation increases the risk of progression to PAD, its severity, and is a predictor of increased mortality in patients with PAD.
  - CRP, IL-6, ICAM-1, TNF-α

- n-3 PUFA can influence both the development and resolution of inflammation, and can improve endothelial function.

- In patients with stable CAD, the n-3 PUFA content has been inversely associated with inflammation as measured with CRP.
Goal

- To determine if the n-3 PUFA content (omega-3 index) is associated with circulating markers of inflammation and endothelial function in patients with PAD.

OMEGA-PAD Cohort: 2011-2012

- Patients referred to the San Francisco VAMC vascular surgery outpatient clinic for evaluation of symptomatic PAD
- Prospective Cohort
- Exclusion criteria: significant renal, hepatic, inflammatory disease or infection.

Predictor: The Omega-3 index

- Predictor: Omega-3 index
  - measure of the red blood cell (RBC) content of the two major long-chain n-3 FAs
    - eicosapentaenoic acid (EPA)
    - docosahexaenoic acid (DHA)
- Expressed as a percentage of total RBC FA
- Marker of tissue n-3 PUFA content and an independent graded risk factor for death from CAD.

Outcome

- Outcomes: Inflammation and vascular function
  - Inflammatory markers
    - CRP
    - IL-6
    - ICAM-1
    - TNF-α
  - Endothelial function
    - Flow-mediated brachial artery vasodilation (FMD)
Statistics

- Linear models to estimate the relationship between decreases in omega-3 index and inflammatory markers (log transformed)
- Results back-transformed to yield percentage changes in the marker for each standard deviation decrease in the omega-3 index
- Covariates: retained if omitting them changed the adjusted coefficient for the omega-3 index by more than 5%

Results

- 74 patients referred for evaluation of PAD agreed to participate in the study
  - 61: claudication
  - 3: critical limb ischemia
  - 10: normal ABI

Demographics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Tertile I</th>
<th>Tertile II</th>
<th>Tertile III</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, Mean ± SD</td>
<td>64 ± 9</td>
<td>61 ± 10</td>
<td>66 ± 8</td>
<td>0.004</td>
</tr>
<tr>
<td>Male, %</td>
<td>51 (39)</td>
<td>51 (52)</td>
<td>60 (78)</td>
<td>0.64</td>
</tr>
<tr>
<td>Caucasian</td>
<td>14 (36)</td>
<td>14 (40)</td>
<td>15 (40)</td>
<td>0.98</td>
</tr>
<tr>
<td>Median CRP (ng/mL)</td>
<td>1.5 (0.6)</td>
<td>3.6 (2.9)</td>
<td>8.1 (9.3)</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Biomarkers

<table>
<thead>
<tr>
<th>Variables</th>
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<th>Tertile II</th>
<th>Tertile III</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean CRP: 5.0 ± 5.0 mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean omega-3 index: 5.0% ± 1.8% (median 4.5%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>146 ± 36</td>
<td>194 ± 36</td>
<td>145 ± 41</td>
<td>0.06</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>59 ± 29</td>
<td>56 ± 17</td>
<td>75 ± 36</td>
<td>0.11</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>171 ± 119</td>
<td>174 ± 144</td>
<td>115 ± 78</td>
<td>0.12</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>3.6 ± 0.3</td>
<td>3.6 ± 0.3</td>
<td>3.6 ± 0.3</td>
<td>0.19</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
<td>0.05</td>
</tr>
<tr>
<td>HbA1c</td>
<td>5.2 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>0.28</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsCRP (mg/dL)</td>
<td>1.4 ± 0.9</td>
<td>1.6 ± 0.9</td>
<td>1.4 ± 0.9</td>
<td>0.01</td>
</tr>
<tr>
<td>fCRP (mg/dL)</td>
<td>0.4 ± 0.4</td>
<td>0.5 ± 0.5</td>
<td>0.2 ± 0.3</td>
<td>0.14</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>2.5 ± 0.3</td>
<td>3.4 ± 0.3</td>
<td>2.4 ± 0.4</td>
<td>0.62</td>
</tr>
<tr>
<td>IL-1RA (pg/mL)</td>
<td>0.5 ± 0.3</td>
<td>0.7 ± 0.5</td>
<td>0.6 ± 0.4</td>
<td>0.39</td>
</tr>
</tbody>
</table>

 rocked chair, was sitting

4/19/2013
**Vascular Function Testing**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>38</td>
<td>33</td>
</tr>
<tr>
<td>IL-6</td>
<td>-0.76</td>
<td>-0.75</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>-0.44</td>
<td>-0.43</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.5</td>
<td>-0.48</td>
</tr>
</tbody>
</table>

**Percent change in inflammatory markers by per standard deviation (1.8%) decrease in the omega-3 index.**

<table>
<thead>
<tr>
<th>Inflammatory marker</th>
<th>% Increase</th>
<th>95% CI</th>
<th>P-value</th>
<th>% Increase</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>38</td>
<td>9.75</td>
<td>.007</td>
<td>33</td>
<td>1.75</td>
<td>.06</td>
</tr>
<tr>
<td>IL-6</td>
<td>-0.76</td>
<td>-0.75</td>
<td></td>
<td>-0.75</td>
<td>-0.74</td>
<td>.0001</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>-0.44</td>
<td>-0.43</td>
<td></td>
<td>-0.43</td>
<td>-0.42</td>
<td>.8</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.5</td>
<td>-0.48</td>
<td></td>
<td>-0.48</td>
<td>-0.46</td>
<td>.71</td>
</tr>
</tbody>
</table>

**Resolution of inflammation in our cohort**

- **n-6 PUFA**
  - DHA Metabolome at Baseline
  - EPA Metabolome at Baseline
- **N-3 PUFA**
  - EPA Metabolome at Baseline

**CRP levels varying by omega-3 index categories**

*Graded for coronary artery disease*
Conclusions

• The omega-3 index was inversely associated with plasma levels of CRP and IL-6

• In our cohort, there were no significant relations with measures of vascular/endothelial function

• Further studies are needed to determine if manipulation of omega-3 index via dietary changes or fish oil supplementation could reduce vascular inflammation and related symptoms
  – OMEGA-PAD Trial

Summary

1. n-3 PUFA have an important effect on endothelial function
   – Inflammation

2. n-3 PUFA have beneficial effects in patients with heart disease

3. More studies are needed to understand the effects of n-3 PUFA in PAD

The future

• New frontiers are being explored in lipidomics with the concept of resolution of inflammation
  – PAD patients, who are at high risk of inflammation is a subset that could potentially draw advantage

• As new investigations and trials take place, encouraging patients to adopt a healthy diet with high intake of fish could be beneficial.