What is the Evidence on Omega-3 and Omega-6 Fatty Acids in the Prevention/Promotion of Atherosclerosis

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How many times a week should we eat fish as per AHA recommendations?
1. Once
2. Twice
3. 3 times
4. Every day
5. There are no recommendations from the AHA on this subject

How many grams of omega-3 fatty acids are recommended for patients with hypertriglyceridemia?
1. 0.5 gm/day
2. 1 gm/day
3. 1-2 gm/day
4. 2-4 gm/day
5. 5 gm/day

Goals
1. Review Current Recommendations
2. Examine Molecular Aspects
3. Review Evidence from CAD and PAD Trials
4. Explore the Omega-6 Controversy
Western Diet and Disease

- About ~10,000 years ago, profound environmental changes related to agriculture and animal husbandry took place.
- Too recent for the human genome to adapt
- Has led to the development of new diseases of our Western civilization

Differences Between Hunter-Gatherer Diets and Western Diets

<table>
<thead>
<tr>
<th></th>
<th>Hunter-Gatherer</th>
<th>Western Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total E intake</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td>Total Carbohydrate</td>
<td>Less</td>
<td>More</td>
</tr>
<tr>
<td>Cereal Grains</td>
<td>Minimal</td>
<td>Substantial</td>
</tr>
<tr>
<td>Fruits/Vegetables</td>
<td>Twice as much</td>
<td>Half as much</td>
</tr>
<tr>
<td>Fibers</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td>Proteins</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td>Total Fat intake</td>
<td>Equal</td>
<td>Equal</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td>w6/w3</td>
<td>1:1</td>
<td>&gt;20:1</td>
</tr>
<tr>
<td>Long-chain essential fatty acids</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td>Free water intake</td>
<td>More</td>
<td>Less</td>
</tr>
</tbody>
</table>

2006 AHA Recommendations: Diet and Lifestyle
- Balance calorie intake and physical activity to achieve or maintain a healthy body weight
- Consume a diet rich in vegetables and fruits
- Choose whole-grain, high-fiber foods
- Consume fish, especially oily fish, at least twice a week
- Limit your intake of saturated fat to <7% of energy, trans fat to <1% of energy and cholesterol to <300mg/day
- Minimize your intake of beverages and foods with added sugars
- Choose and prepare foods with little or no salt.
- If you consume alcohol, do so in moderation
- When you eat food that is prepared outside of the home, follow the AHA diet and lifestyle recommendations.


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>

Fish 101. www.americanheart.org

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   - Hypertriglyceridemia: 2-4gm/day
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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

\[ \text{Methyl carbon} \quad 1 \quad 9 \quad 6 \quad 16 \quad 3 \quad 12 \quad 9 \quad 1 \]
\[ \text{Carbonyl carbon} \quad 1 \quad 14 \quad 12 \quad 6 \quad 10 \quad 15 \]
Essential Fatty Acid Families

**ω-6 family**

- C18:2 ω-6: Linoleic
  - Corn Oil
  - Safflower Oil
  - Sunflower Oil

- C20:4 ω-6: Arachidonic
  - Poultry
  - Meats

**ω-3 family**

- C18:3 ω-3: α-Linolenic
  - Flaxseed Oil
  - Canola Oil
  - Soybean Oil

- C20:5 ω-3: Eicosapentaenoic (EPA)
  - Oily Fish
  - Fish Oil Capsules

- C22:6 ω-3: Docosahexaenoic (DHA)
  - Corn Oil
  - Safflower Oil
  - Sunflower Oil

More thrombotic and inflammatory metabolites

Less thrombotic and inflammatory metabolites

Postulated Effects of Omega-3

- 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)
  - 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
  - reduced NF-κB system of transcription factors (this controls the coordinated expression of adhesion molecules and leukocyte-specific chemoattractants upon cytokine stimulation)
  - Reduced monocyte cell adhesion to cytokine-activated endothelium

Food content of Fatty Acids

<table>
<thead>
<tr>
<th></th>
<th>Omega-2</th>
<th>Omega-3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPA</td>
<td>DHA</td>
</tr>
<tr>
<td>corn oil</td>
<td>5000</td>
<td>3600</td>
</tr>
<tr>
<td>canola oil</td>
<td>30000</td>
<td>1330</td>
</tr>
<tr>
<td>soybean oil</td>
<td>58000</td>
<td>7600</td>
</tr>
<tr>
<td>sunflower oil</td>
<td>60200</td>
<td>300</td>
</tr>
<tr>
<td>safflower oil</td>
<td>17000</td>
<td>1090</td>
</tr>
<tr>
<td>sunflower oil</td>
<td>34300</td>
<td>1000</td>
</tr>
<tr>
<td>flaxseed</td>
<td>14300</td>
<td>610</td>
</tr>
<tr>
<td>cod liver oil</td>
<td>295000</td>
<td>250</td>
</tr>
<tr>
<td>fish oil</td>
<td>14800</td>
<td>530</td>
</tr>
<tr>
<td>wheat</td>
<td>34100</td>
<td>390</td>
</tr>
</tbody>
</table>

With permission from Harris WS

Treatment of endothelial cells with Fatty Acids: 4 hours

Grenon et al. CSVS Meeting, October 2010
Adhesion of monocytes to endothelial cells with fluorescence microscopy

**Control**

**IL-1B (10 ng/ml)**

**AA (5 ng/ml)**

Grenon et al. CSVS Meeting, October 2010

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**Fatty Acids on inflammation and adhesion molecules in endothelial cells**

**IL-6**

**iCAM**

**VCAM**

Grenon et al., manuscript in preparation

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**Goals**

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2. **Examine Molecular Aspects**
   - Omega-3 and Omega-6 FAs have different molecular structures which confer different biological properties. These likely impact processes involved in the development of atherosclerosis.
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**Potential EPA and DHA Effects**

- Reduced atherosclerosis
- Antiarrhythmic 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

Massaro et al. Cell & Mol Biol 2010
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

_Burr et al, Lancet 1989_

DART results

- 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.

_Gissi Prevenzione Investigators, Lancet 1999_

Gissi Trial

- Omega-3 FAs reduced the primary endpoint by 15% (p=0.023).
- Mortality was significantly lowered after 3 months of treatment
- Sudden death was significantly decreased starting at 4 months
- Decreased in cardiovascular, cardiac and coronary deaths were observed after 6-8 months of treatment
- Combined treatment with omega-3 FAs and Vitamin E led similar results to omega-3 FAs alone.
Jelis Trial (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

JELIS trial: Reduction in MCE

PAD subgroup- JELIS trial

Ishikawa et al, Circ J 2010

Most Recent Meta-Analysis (2009)
- Most recent meta-analysis on omega-3 FAs
- 11 prospective, randomized, placebo-controlled clinical trials
- 39,044 patients with recent MI, with an implanted cardioverter defibrillator, patient with HF, PAD and hypercholesterolemia.
- Dose used 1.8gm/day for a duration of 2.2 +/- 1.2 years
- Overall reduction in the risk of
  - cardiovascular death
  - SCD
  - all-cause mortality
  - nonfatal cardiovascular events

Marik and Varon, Clin Cardiol 2009
The OMEGA trial

- First randomized study on the effects of highly purified omega-3 acid ethyl ester (1gm/day for 1 year) in addition to current guideline therapy 3-14 days after MI
- 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

Since the pathogenesis of coronary heart disease and peripheral arterial disease (PAD) is similar, could omega-3 fatty acids also be effective in the treatment and prevention of PAD?

Eckel RH, Circulation 2010
Summary of PAD studies

**Cochrane Review 2007**

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

**Issues...**

- Only advantage appears to be 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

**The Effects of Omega-3 Fatty Acids Supplementation on Endothelial Function and Inflammation (OmegaPAD)**

- Randomized, double-blinded, placebo-controlled trial
- "OmegaPAD"
- Team:
  - PI: SM Grenon
  - Co-I: MS Conte, C Owens, J Rapp
- Specific Aims:
  - To test the impact of high-dose, short-duration dietary omega-3 supplementation on endothelial function in patients with PAD.
  - To determine if high-dose, short-duration dietary omega-3 supplementation reduces levels of vascular inflammation in patients with PAD.
- Population:
  - N=80, claudicants at the SF VAMC and UCSF
Design and Outcomes

Endpoints
- Endothelial Function
  - FMD
- Inflammatory profile
  - hsCRP
  - IL-6
  - sICAM-1
  - 15-epimeric lipoxin
- Demographics
- Omega-3 index
- Nutritional Survey

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   - CAD: Significant Level I evidence
   - PAD: Evidence is lacking, studies undergoing

4. Explore the Omega-6 Controversy

Area of Controversy: Omega-6 Fatty Acids

“Finally, the view that all n-6 PUFA are proinflammatory requires revision, in part, and their essential regulatory and developmental role in the immune system warrants appreciation.”


Old tale or the new tale… inflammation

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

Stables and Gilroy. Prog Lipid Res 2011
New AHA Recommendations

“Consumption of at least 5-10% of energy from omega-6 PUFA reduces the risk of CHD relative to lower intakes, and higher intakes may be even more beneficial. We have no clinical evidence of harm. To recommend reductions in omega-6 PUFA intakes from their current levels would be more likely to increase than decrease risk for CHD.”


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4. Explore the Omega-6 Controversy
   - Exploding world of new molecules being discovered, which may shift (or not) our views on nutrition for patients with atherosclerosis.
Conclusions

- Fish oil has possibly great health benefits with very low side-effects
  - Worth taking…

- The impact of fish oil in PAD remains to be determined

- The omega-6 FAs are essential nutrients with novel anti-inflammatory properties
  - The story remains to be told…

Acknowledgements

- Dr. Millie Hughes-Fulford
- Dr. Michael Conte
- Dr. Christopher Owens
- Dr. Joseph Rapp
- Dr. William Harris

“Fish oil is a whale of a story, that not surprisingly gets bigger with every telling.”

~J A Rogans, New England Journal of Medicine, 1996
Fish Oil Side-Effects

- Most common is a fishy aftertaste.
- In the GISSI Prevention study
  - 0.85 g of omega-3 fatty acids per day for 3.5 years
  - 3.8% of patients discontinued taking their supplements (compared with 2.1% for the vitamin E group).
  - Gastrointestinal disturbances (4.9%) and nausea (1.4%) were the most commonly reported side effects.
- HARP Research Group study
  - Twelve capsules containing 6 g of omega-3 fatty acids were fed to 41 patients for 2.4 years.
  - 3 patients dropped from the study claiming intolerance to the capsules.
- A study by Leaf et al.
  - 6-month trial providing 275 patients with 5.9 g of EPA/DHA in 16 capsules daily.
  - There was no difference between the fish-oil and corn-oil control groups for any adverse event.
  - Gastrointestinal upset was reported by 8% of the latter and 7% of the former.
- A study by Chm Schacky et al.
  - Fish oil was given at the dose of 4 g/day for 3 months, then 3 g/day for 21 months (112 patients).
  - 3 patients had mild gastrointestinal discomfort.
  - 1 patient had a itchy rash (unlikely related to the study medication as per the authors).
  - Minor hematoma after the second angioplasty was reported.

Fish Content

- Table 1: Fish Content of EPA and DHA

<table>
<thead>
<tr>
<th>Type</th>
<th>EPA (g)</th>
<th>DHA (g)</th>
<th>EPA + DHA (g)</th>
<th>EPA/DHA Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>2.08</td>
<td>1.65</td>
<td>3.73</td>
<td>1.25</td>
</tr>
<tr>
<td>Corn</td>
<td>0.00</td>
<td>0.10</td>
<td>0.10</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Timing of Clinical benefits

- Table 2: Major Classes of Fatty Acids

<table>
<thead>
<tr>
<th>Type</th>
<th>Fatty Acids</th>
<th>Formula</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>I omega-6</td>
<td>Linoleic acid</td>
<td>C18:2</td>
<td>Most vegetable oils (corn, cotton, animal fats)</td>
</tr>
<tr>
<td>II omega-3</td>
<td>Arachidonic acid</td>
<td>C20:4</td>
<td>Poultry, meat</td>
</tr>
<tr>
<td>III omega-3</td>
<td>docosahexaenoic acid</td>
<td>C22:6</td>
<td>Substantial vegetable oils (canola, corn)</td>
</tr>
<tr>
<td>IV saturated fats</td>
<td>Palmitic acid</td>
<td>C16:0</td>
<td>Animal and vegetable fats</td>
</tr>
<tr>
<td>V unsaturated fats</td>
<td>Stearic acid</td>
<td>C18:0</td>
<td>Butter, palm oil, lard oil, coconut oil, oral animal fats</td>
</tr>
</tbody>
</table>

*The omega number refers to the position of the first double bond from the methyl end of the molecule. (The notation shows the total number of carbon atoms and total number of double bonds. Adapted with permission from Lavie et al.)*
Controversy: Omega-6 FAs

- **Eat Less**
  - LA plays a key role in the inflammatory process (by raising AA)
  - Lowering LA ratio will increase tissue levels of EPA and DHA
  - Since aspirin reduces the risk of CAD (in part by reducing the effects of AA), eating less LA should reduce CAD.

- **Eat More**
  - Few randomized trials demonstrate a possible improvement in CAD
  - Variations in LA intake does not significantly affect AA tissue levels (0.2% conversion)
  - AA can be converted into different molecules (both pro- and anti-inflammatory)

2.11.11 Adhesion Assay

- Treatment of ECs with FA, TNF and IL1b at final concentrations of 5ug/ml, 100ng/ml and 10ng/ml, respectively.
- 24 hour FA and 4 hour Cytokine incubation.
- 4 hour FA and cytokine incubation. (mixed together)
- 20 minute incubation with monocytes.

- Regular 96-well plates.
- BSA was used.
- One PBS wash between replacing FA and cytokine containing media to monocyte containing media.
- Only one final wash with PBS before reading with Floro

qPCR Data

- ECs cells grown to confluency.
- Treated with FAs and Cytokines
- Harvested and RNA extracted.
- Reverse Trascription to make DNA
- Tested with hCPII, hIL-6, hICAM, hVEGF, hVCAM, hTGFb1 and hTNFa.
- 24 hour FA, followed by a 4h Cytokine incubation.