Plant versus Marine n-3 fatty acid and selected CVD risk factors

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5th International Congress on Vegetarian Nutrition
March 4-6th, 2008
Loma Linda University
CVD and n-3 PUFA

• The role of n-3 PUFA in preventing CVD is well established

• One of the compelling public health messages to emerge for prevention of CVD

*Increase consumption of n-3 PUFA in the daily diet*
CVD and n-3 PUFA

- Both 18:2 ALA and longer chain 20:5 EPA/22:6 DHA intake correlated with reduced risk of CVD

- Evidence
  - Epidemiological and clinical trials
  - Primary and secondary prevention
RCT for secondary prevention of all-cause mortality, Wang 2006
RCT for secondary prevention of cardiac death, Wang 2006
RCT for secondary prevention of nonfatal MI, Wang 2006
Cohort studies: primary prevention, Wang 2006

• ↓ in all-cause mortality with increased fish intake

• ↓ in fatal and total CHD with higher fish consumption

• ↓ reduction in risk of MI with increasing fish intake
Multivariate RR of fatal IHD
Hu, 1999
### N-3 PUFA: Inflammation and Endothelial activation, Lopez-Garcia, 2004

<table>
<thead>
<tr>
<th></th>
<th>ALA</th>
<th>EPA + DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age-adjusted</td>
<td>Multivariate-adjusted$^3$</td>
</tr>
<tr>
<td>Log CRP, mg/L</td>
<td>-0.25 (0.20)</td>
<td>-0.55 (0.02)</td>
</tr>
<tr>
<td>Log IL-6, ng/L</td>
<td>-0.28 (0.02)</td>
<td>-0.36 (0.01)</td>
</tr>
<tr>
<td>Log sTNFR-2, μg/L</td>
<td>-0.13 (0.08)</td>
<td>-0.14 (0.14)</td>
</tr>
<tr>
<td>Log E-selectin, ng/L</td>
<td>-0.13 (0.07)</td>
<td>-0.24 (0.008)</td>
</tr>
<tr>
<td>Log sICAM-1, μg/L</td>
<td>-0.06 (0.15)</td>
<td>-0.07 (0.18)</td>
</tr>
<tr>
<td>Log sVCAM-1, μg/L</td>
<td>-0.06 (0.17)</td>
<td>-0.08 (0.14)</td>
</tr>
</tbody>
</table>
# Dietary sources of ALA

<table>
<thead>
<tr>
<th>Sources of ALA</th>
<th>ALA (g/tbsp or 28 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumpkin seeds</td>
<td>0.051</td>
</tr>
<tr>
<td>Olive oil</td>
<td>0.103</td>
</tr>
<tr>
<td>Walnuts, black</td>
<td>0.156</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>1.231</td>
</tr>
<tr>
<td>Rapeseed oil</td>
<td>1.302</td>
</tr>
<tr>
<td>Walnut oil</td>
<td>1.414</td>
</tr>
<tr>
<td>Flaxseeds</td>
<td>2.350</td>
</tr>
<tr>
<td>Walnuts, English</td>
<td>2.574</td>
</tr>
<tr>
<td>Flaxseed oil</td>
<td>7.249</td>
</tr>
</tbody>
</table>
## Dietary sources of EPA/DHA

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Quantity</th>
<th>EPA +DHA (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuna, canned</td>
<td>3 oz cooked</td>
<td>733</td>
</tr>
<tr>
<td>Shrimp</td>
<td>3 oz cooked</td>
<td>267</td>
</tr>
<tr>
<td>Salmon</td>
<td>3 oz cooked</td>
<td>1825</td>
</tr>
<tr>
<td>Microalgae oil</td>
<td>3 capsules</td>
<td>360</td>
</tr>
<tr>
<td>Seaweed, Kelp, raw</td>
<td>100 g</td>
<td>4</td>
</tr>
<tr>
<td>Seaweed, Laver, raw</td>
<td>100 g</td>
<td>80</td>
</tr>
<tr>
<td>Seaweed, Wakame, raw</td>
<td>100 g</td>
<td>190</td>
</tr>
<tr>
<td>DHA-enriched egg</td>
<td>1 egg</td>
<td>114</td>
</tr>
</tbody>
</table>
Intake of n-3 PUFA

• NHANES data 1999-2000

• EPA + DHA intake 110-170 mg/d
  – Primary source (Tuna > shrimp > salmon)
  – Recommended 500-800 mg/d

• ALA intake 1.3-1.7 g/d
  – Recommended 1.4-1.8 g/d
AHA versus EPA/DHA Review

• Subjects
  – Mildly dyslipidemic, n=57, 35-60 y (2005)
  – Hypercholesterolemic, n=37, 60-78 y (2006)
AHA versus EPA/DHA Review

- **Study Design**
  - Controlled, parallel study, free living
  - 6 weeks, 12 weeks or 6 mo
  - Experimental shortening used
    - Flaxseed mostly used as source of ALA
AHA versus EPA/DHA review

• **Diets**
  – Absolute amount of ALA (4.5 g, 9.5 g)
  – Absolute amount of EPA/DHA (0.8 g, 1.6 g)

  – ALA diet (6.8 g/d); EPA/DHA diet (1.6 g/d)

  – Ratio of n-6:n-3 (28:1; 5:1; 0.5:1)
AHA versus EPA/DHA Review

• Outcomes
  – No change to ↓ LDL-C (ALA)
  – No change to ↓ TAG (EPA/DHA)
  – No change to ↑ HDL (EPA/DHA)
Walnuts versus Fatty fish?

• Studies comparing whole foods rich in ALA versus EPA/DHA side by side is sparse especially at doses recommended for primary prevention

• For both fatty fish and walnut consumption public health recommendations exist for prevention of heart disease
# AHA recommendations for n-3 PUFA

<table>
<thead>
<tr>
<th>Population</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients without documented CHD</td>
<td>Eat a variety of oily fish, 2 x/wk, include oils and foods rich in $\alpha$-linolenic acid (flaxseed, walnuts, canola, and soybean oils)</td>
</tr>
<tr>
<td>Patients with documented CHD</td>
<td>Consume $\approx 1$ g of EPA + DHA/d, preferably from oily fish or supplements.</td>
</tr>
<tr>
<td>Patients needing triglyceride lowering</td>
<td>Two to four grams of EPA + DHA per day provided as capsules under a physician's care.</td>
</tr>
</tbody>
</table>
Fatty fish intake

• Include at least 2 servings of fatty fish per week (AHA, 2006)
  – 0.5-1.8 g/d EPA+DHA
  – 4 ounce serving size
  – Variety of fatty fish
Walnut intake

• Eat 1.5 oz/d of walnuts as a part of low saturated fat, low cholesterol diet without increasing calories (FDA qualified health claim, 2004)

  – 1 ounce is 28.4 g
Walnuts versus Fatty fish?

• Data on other CVD risk factors are inconsistent and sparse

• Methodological rigor not seen in many studies comparing ALA versus EPA/DHA
Fatty fish: Blood lipids
Review

• ~ 2-4 g/d of EPA+DHA

• ↓ triglyceride (25-30%)

Doses < 1 g/d inconsistent effects

More effective in hypertriglyceridemic than healthy subjects
Walnuts: Blood lipids Review

- Healthy to moderately hyperlipidemic
- Controlled parallel to crossover design
- Free living to feeding study
- Comparison diet: low fat to Mediterranean
Walnuts: Blood lipids Review

- Walnuts: 40-84 g/d (1.5-3 oz/d)
- 3-6 week intervention
- TC ↓ (4-12%)
- LDL-C ↓ (6-16%)

Both in healthy and hypercholesterolemic
To compare plant (walnuts, ALA) versus marine (salmon, EPA/DHA) source of n-3 PUFA at doses recommended for primary prevention on selected CVD risk factors in normal to mildly dyslipidemic individuals.
Study Design

- Randomized crossover feeding trial
- One week Western-type diet containing 34% energy from fat (run-in period)
- Three isoenergetic diets for four weeks each
Subjects

• Ages 23-65 y, men and women, n=25

• Baseline values
  – Mean LDL-C: 3.53 (1.82-5.66 mmol/L)
    • 136 mg/dL (70-219 mg/dL)
  – Mean triglyceride: 1.25 (0.66-3.33 mmol/L)
    • 110 mg/dL (58-294 mg/dL)
Diet design

- <30% energy from fat; <8-9% en from SFA; < 300 mg/d cholesterol

Control diet (exclude nuts, fatty fish)
Walnut diet (42.5 g/2400 kcal, 6 d/wk)
Fish diet (2 servings or 226 g/wk)
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Control Diet</th>
<th>Fish Diet</th>
<th>Walnut Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate, g</td>
<td>347</td>
<td>347</td>
<td>359</td>
</tr>
<tr>
<td>Protein, g</td>
<td>87</td>
<td>89</td>
<td>87</td>
</tr>
<tr>
<td>Fat, g</td>
<td>79</td>
<td>78</td>
<td>83</td>
</tr>
<tr>
<td>SFA, g</td>
<td>25</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>MUFA, g</td>
<td>36</td>
<td>35</td>
<td>27</td>
</tr>
<tr>
<td>Trans fat, g</td>
<td>2.7</td>
<td>2.5</td>
<td>2.2</td>
</tr>
<tr>
<td>LA, g</td>
<td>10</td>
<td>9.5</td>
<td>24</td>
</tr>
<tr>
<td><strong>ALA, g</strong></td>
<td><strong>1</strong></td>
<td><strong>1.1</strong></td>
<td><strong>4.7</strong></td>
</tr>
<tr>
<td>LA:ALA ratio</td>
<td>10:1</td>
<td>10:1</td>
<td>4.8:1</td>
</tr>
<tr>
<td><strong>EPA + DHA, g</strong></td>
<td><strong>0.04</strong></td>
<td><strong>0.8</strong></td>
<td><strong>0.04</strong></td>
</tr>
</tbody>
</table>
## Erythrocyte membrane fatty acid

<table>
<thead>
<tr>
<th>Fatty acid, mol %</th>
<th>Control</th>
<th>Fish</th>
<th>Walnut</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA</td>
<td>38.9 (0.39)</td>
<td>40.2 (0.39)</td>
<td>39.9 (0.39)</td>
<td>0.0409</td>
</tr>
<tr>
<td>MUFA</td>
<td>15.8 (0.19)</td>
<td>15.8 (0.19)</td>
<td>14.5 (0.19)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>PUFA</td>
<td>38.6 (0.38)</td>
<td>39.4 (0.38)</td>
<td>41.0 (0.38)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>LA (18:2 n-6)</td>
<td>10.5 (0.29)</td>
<td>9.7 (0.29)</td>
<td>12.4 (0.29)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ALA (18:3 n-3)</td>
<td>0.13 (0.01)</td>
<td>0.12 (0.01)</td>
<td>0.26 (0.01)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>EPA (20:5 n-3)</td>
<td>0.51 (0.04)</td>
<td>1.08 (0.04)</td>
<td>0.58 (0.04)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>DHA (22:6 n-3)</td>
<td>4.52 (0.21)</td>
<td>5.12 (0.21)</td>
<td>4.46 (0.21)</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
Blood Lipids

• Walnut Diet
  – Total Cholesterol ↓ (5.4%)
  – LDL-C ↓ (9.3%)
  – Apo B ↓
  – LDL: HDL ↓

• Fish Diet
  – LDL-C ↑
  – HDL-C ↑
  – TAG ↓ (11%)
Total and LDL cholesterol

<table>
<thead>
<tr>
<th>Treatment Diet</th>
<th>Total Cholesterol, mmol/L</th>
<th>LDL-C, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Diet</td>
<td>5.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Fish Diet</td>
<td>5.50</td>
<td>3.50</td>
</tr>
<tr>
<td>Walnut Diet</td>
<td>4.80</td>
<td>3.20</td>
</tr>
</tbody>
</table>
Triglyceride and apo B

Control Diet
Fish Diet
Walnut Diet

Triglyceride, mmol/L
Apo B, g/L

Treatment Diet
HDL and apo A-1

![Bar chart showing HDL-C and apo A1 levels under different diets]

- Control Diet
- Fish Diet
- Walnut Diet

- HDL-C, mmol/L
- Apo A1, g/L
Cholesterol lowering effects of walnuts

- PUFA
  - ALA-2.6 g
  - LA-10.8 g
- Fiber (1.9 g)
- Phytosterols (40 mg)
Cholesterol lowering effect

- ↓ SFA/PUFA ratio alters membrane fluidity (LA + ALA)
- Receptor mediated uptake of LDL ↑
- ↓ Plasma LDL-C
Triglyceride lowering effect
ALA versus EPA/DHA

- Lack of triglyceride lowering effect of ALA

- Limited by DHA levels in membrane PL?
  - Altering n-6: n-3 ratio
    - Increasing ALA
    - Decreasing LA
Increasing ALA
Arterburn, 2006
Increasing ALA
Arterburn, 2006
Increasing ALA
Arterburn, 2006
Decreasing LA
Liou, 2007
Decreasing LA
Liou, 2007
Eicosanoids

Walnut diet vs. Control diet
Fish diet vs. Control diet
Walnut diet vs. Fish diet
Endothelial Adhesion

![Graph showing percentage difference for sICAM-1 and sE-selectin](image)
Inflammation

• No differences between diets in inflammatory markers studied
  – CRP, IL-6, IL-1β, TNF-α

• Non-inflammatory state at baseline
Mechanism (Calder PC, AJCN 2006)

Diagram showing the mechanism of action of DHA and EPA on the production of prostaglandins and leukotrienes, with the inhibition of COX-2 and 5-LOX enzymes.
Walnuts versus fatty fish

- Similar effects on eicosanoids, adhesion molecules

- Depend on ALA conversion to EPA

- Other components of walnuts?
  - $\gamma$ tocopherol (5.9 mg)
  - Arginine (0.65 g)
  - Ellaigic acid
Influence of membrane EPA
Zhao G, 2007
Influence of background diet
Paschos GK, 2004
Influence of absolute amount of n-3 PUFA, Mantzioris E, 2007

<table>
<thead>
<tr>
<th></th>
<th>Duration of diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 wk</td>
</tr>
<tr>
<td>TXB₂ (nmol/L)</td>
<td>34.9 ± 17.6ᵃ</td>
</tr>
<tr>
<td>PGE₂ (nmol/L)</td>
<td>15.3 ± 11.6ᵃ</td>
</tr>
<tr>
<td>IL-1β (µg/L)</td>
<td>40.93 ± 12.17ᵃ</td>
</tr>
<tr>
<td>TNF-α (pmol/L)</td>
<td>680 ± 309ᵃ</td>
</tr>
</tbody>
</table>
Study Summary

• Walnuts and Fatty fish do not have similar effects on blood lipids and selected markers of endothelial adhesion and thrombosis

• Synergistic effect of walnuts and fatty fish may exist
Public Health Relevance

• Vegetarian do not eat fish

• They can ↑ ALA intake by including foods such as walnuts or other sources (flaxseed, canola oil)

• They may need to consider obtaining EPA/DHA through other options
Public Health Relevance

- Non-vegetarians may eat fish, but currently it does not meet EPA/DHA levels that are recommended.

- Intake of ALA also need to be ↑ because the favorable effects seen with ALA not exhibited by EPA/DHA.
Future Directions

• Large clinical trials for the primary prevention of CVD with both ALA and EPA/DHA

• Dose response data on ALA, EPA, DHA
Future Directions

• More systematic studies on newer markers of CVD risk for primary prevention

• Comparison of different sources of ALA with respect to newer CVD risk factors especially
Acknowledgement

• Ella Haddad, DrPH, MS, RD
• Joan Sabaté, MD, DrPH
• Alfredo Mejia, DrPH, RD
• Yu-Lan Chiang, DrPH student
• Diana Torres, MPH student