Applying Clinical Precision to Fatty Acid Assessment and Therapeutics

Michael A. Schmidt, Ph.D.
Chairman, MetaboLogics, LLC
Co-Chair, Advanced Pattern Analysis and Countermeasures Group
Principal Investigator, NASA Ames Research Center
Girl Age Six: Bullet Wound to the Abdomen
• Numbness of the hands and feet
• Loss of sensation
• Leg pain
• Blurred vision
• Tremors of the arm
• Decreased vibration sense
• Frequent episodes of weakness
• Unable to walk for ten to fifteen minutes at a time

Signs of nerve degeneration

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After fatty acid ratios restored...

“All neurological symptoms disappeared.”

Omega-6:Omega-3 Ratio
DEFINES Human Structure/Function

Omega-6:Omega-3 Ratios in Selected Cases

<table>
<thead>
<tr>
<th>Omega-Content Per Circumstance</th>
<th>Relative Omega-6:Omega-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPN Formula</td>
<td>115</td>
</tr>
<tr>
<td>Revised TPN</td>
<td>6</td>
</tr>
<tr>
<td>Ideal</td>
<td>4</td>
</tr>
<tr>
<td>Modern Diet</td>
<td>30</td>
</tr>
<tr>
<td>Breastmilk (some)</td>
<td>60</td>
</tr>
</tbody>
</table>

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Key Objectives

• A simple insight into the roles of fatty acids & phospholipids?
• What are the specific roles of lipids in membrane architecture?
• What are the important molecular forms?
• How do these forms shape metabolic fitness and performance?
• Appropriate laboratory tests?
• How do I interpret the fatty acid patterns?
• What are the key decision points in using FA in prevention & therapeutics?
A Simple Insight
Speed of Processing: Signature Component of Brain Fitness

Performance declines with increasing age for Speed of Processing, Working Memory, and Long-Term Memory.

Performance is preserved over age for World Knowledge.

The Biological Imperative

1 trillion glial cells

100 billion neurons

Uses 25% of body’s energy

3 pounds

60% fat

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The Biological Imperative: High Energy Cost of Processing Fat

60% fat

Processing FA and PL uses 26% of net brain ATP consumption
Metabolic Fitness: The Biological Imperative

Membrane Lipids

- Processing
- Brain Fats = 26% of net brain ATP
- N-3 lipids an imperative for metabolic fitness
- Increased physical/cognitive load requires increased precursors

Structural
Signaling
Repair-Resolving
Gene Transcription Factors

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Structural Roles of Lipids in Membrane Architecture
Surface Area of PL Membranes

Baker, S et al. Yale U

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Phospholipid Architecture

- Polar "head" containing phosphate
- Nonpolar "tail" containing hydrocarbon

Inside cell

Outside cell

Neuroscience: Exploring the Brain, 3rd Ed. Bear, Connors, and Paradiso Copyright © 2007 Lippincott Williams & Wilkins

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Phospholipids Dominate the Cell Membrane

*Phosphatidylcholine* accounts for >50% of phospholipids in cell membranes
Brain Essential Fatty Acid Composition
Ethanolamine Phosphoglycerides


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Myelin Heavily Dependent upon Fat

Min Y et al. PNAS 2009;106:3154-3159

©2009 by National Academy of Sciences

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What are the Important Molecular Forms?
Phospholipid vs.
Triglyceride vs.
Ethyl Ester

Bound forms of EPA and DHA
Molecular Forms of Omega-3’s

**Triglyceride:**
This is the most commonly sold form of fish oils, wherein one or more of the fatty acid positions contain EPA or DHA.

**Phospholipid:**
A phosphate group attached to a glycerol backbone, with two bound fatty acids. Marine phospholipids are unique in that they may contain one or more omega-3 fatty acids. Phospholipids are a major component of ALL cell membranes.

**Ethyl Ester:**
Most commonly associated with Omega concentrates, where the fatty acids are split from the glycerin backbone to allow it to be more easily concentrated via distillation.
Major Phospholipid Classes

Vs.

Triglyceride Backbone

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Phospholipid

EPA or DHA
Also AA (marine)

LA or LNA (plants)
Triglyceride

EPA or DHA

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Varied Fatty Acids Occupy sn-1 and sn-2: In Brain, Omega-3 Fatty Acids Crucial

Fatty Acids can be Cleaved
EPA and DHA can be moved to make omega-3-enriched phospholipids

And replace shorter fatty acids here...

Long Chain Fatty Acids can be Cleaved
Highly Enriched Natural
Omega-3-Enriched Phospholipids

EPA and DHA can be enriched at both positions
To make phospholipids ideal for the retina or the brain

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Modified Head Group: Serine Phospholipids

Polar head can be altered to make EPA or DHA serine phospholipids
**Important Dietary Fatty Acids in Membranes**

- **linoleic acid** 18:2ω-6
- **γ-linolenic acid** GLA 18:3ω-6
- **Dihomo-γ-linolenic** acid DGLA 20:3ω-6
- **arachidonic acid** AA 20:4ω-6
- **docosatetraenoic acid** DTA 22:4ω-6
- **docosapentaenoic acid** DPA 22:5ω-6
- **α-linolenic acid** 18:3ω-3
- **stearidonic acid** 18:4ω-3
- **eicosatetraenoic acid** ETA-3 20:4ω-3
- **eicosapentaenoic acid** EPA-3 20:5ω-3
- **docosapentaenoic acid** DPA 22:5ω-3
- **docosahexaenoic acid** DHA 22:6ω-3

### Synthesis Pathways

- **Δ6 desaturase**
- **Δ5 desaturase**
- **Δ4 desaturase**

### Sources

- **Fish (cold)**
  - Eggs
  - Algae
  - Krill
  - Squid
- **Fish (warm)**
  - Squid
  - Algae
- **Eggs**
- **Red meat**
- **Dairy**
- **Sesame**
- **Corn**
- **Sunflower**
- **Safflower**

### Metabolites

- **pgd<sub>2</sub>, pge<sub>2</sub>, pgf<sub>2α</sub>, pgi<sub>2</sub>, txa<sub>2</sub>, lta<sub>4</sub>, ltb<sub>4</sub>, ltc<sub>4</sub>, lte<sub>4</sub>
- **pgd<sub>3</sub>, pge<sub>3</sub>, pgf<sub>3α</sub>, pgi<sub>3</sub>, txa<sub>3</sub>, lta<sub>5</sub>, ltb<sub>5</sub>, ltc<sub>5</sub>, lte<sub>5</sub>
- **A/J-Ring Neuroprostane 17S Resolvins blocks prostanoids**

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Fatty Acid Type & Membrane Fluidity

(b) Membrane fluidity

Unsaturated hydrocarbon tails with kinks
Saturated hydrocarbon tails
Fatty Acid Type & Membrane Fluidity:
Effect on Receptor Conformation

Dopamine D1 Receptor. Molecular Pharmacology 1999;56(4):675-683
Fatty Acids and the Resolving Process

Fatty Acids and the Resolving Process

Serhan, CN, Chiang, N. British Journal of Pharmacology (2007), 1–16
DHA Effects: New Mechanism

Does DHA Protect Against Trauma Sequelae?

- Five groups of 16 adult male Sprague-Dawley rats
- Subjected to an impact acceleration traumatic brain after having received a prior administration of DHA in doses of 3, 12, and 40 mg/kg for 30 days prior.
- Following sacrifice 1 week after injury, brainstem white matter tracts underwent fluorescent immuno-histochemical processing for labeling of beta amyloid precursor protein (APP), an anatomical marker of brain injury, as well as measurements of CD68 and caspase-3 levels, and water maze testing was used for behavioral assessment.

DHA supplementation resulted in increased serum DHA levels proportionate with the escalating dosage.

Significantly (P < .05) decreased numbers of APP (amyloid precursor protein) levels in all groups of animals receiving pre-injury supplementation with DHA of:

- 4 mg/kg (13,955 axons per mm³)
- 12 mg/kg (4,186 axons per mm³)
- 40 mg/kg (2,827 axons per mm³)

This vs. 37,442 axons per mm³ in unsupplemented animals,

High dose group (40 mg/kg): significantly (P < .05) decreased numbers of APP positive axons, at 1.15 axons per high power field vs. 6.78 in unsupplemented animals.

DHA and Suicide: US Military

- 2002 to 2008
- U.S. military: 800 deaths by suicide vs. 800 controls
- Risk of suicide death was 14% higher per SD of lower DHA percentage (OR = 1.14; 95% CI, 1.02–1.27; P < .03) in adjusted logistic regressions.
- Among men, risk of suicide death was 62% greater with low serum DHA status (adjusted OR = 1.62; 95% CI, 1.12–2.34).

78 U.S. active duty military for EPA/DHA status
Mean index for these military subjects was 3.5%
Significantly below US mean: U.S. (4.5%)
Well below the ideal target of 8-10%.
In this cohort, a low omega-3 index was also associated with:
  • lower cognitive flexibility
  • lower executive function.

Omega-3 Laboratory Tests

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Fatty Acids – RBC/Plasma

- **Polyunsaturated Omega-3**
  - alpha-Linolenic
  - Eicosatetraenoic acid (ETA-3)
  - Eicosapentaenoic
  - Docosapentaenoic
  - Docosahexaenoic

- **Polyunsaturated Omega-6**
  - Linoleic
  - gamma-Linolenic
  - Dihomogammarlinolenic
  - Eicosadienoic
  - Arachidonic (ETA-6)
  - Docosapentaenoic
  - Docosadienoic
  - Docosatetraenoic

- **Polyunsaturated Omega-9**
  - Mead

- **Monounsaturated (Omega-7, Omega-9)**
  - Myristoleic
  - Palmitoleic
  - Vaccenic
  - Oleic
  - 11-Eicosanoic
  - Erucic
  - Nervonic

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Fatty Acids – RBC/Plasma

- **Saturated Fatty Acids (even numbered)**
  - Capric
  - Lauric
  - Myristic
  - Palmitic
  - Stearic
  - Arachidic
  - Behenic
  - Lignoceric
  - Hexacosaenoic

- **Saturated Fatty Acids (odd numbered)**
  - Pentadecanoic
  - Heptadecanoic
  - Nonadecanoic
  - Heneicosanoic
  - Tricosanoic

- **Trans Fatty Acids**
  - Palmitelaidic
  - Elaidic
  - Total C:18 trans

- **Fatty Acid Ratios**
  - Linoleic Acid/Dihomogammalinolenic acid
  - Eicosapentaenoic Acid/Dihomogammalinolenic acid
  - Arachidonic Acid/Eicosapentaenoic acid
  - Triene/Tetraene
### Fatty Acids - Plasma

Ranges are for ages 13 and over.

<table>
<thead>
<tr>
<th><strong>Polyunsaturated Omega-3</strong></th>
<th>Results (µM)</th>
<th>Percentile Ranking by Quintile</th>
<th>95% Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Alpha Linolenic (18:3n3)</td>
<td>45</td>
<td>1st 20%</td>
<td>22 - 144</td>
</tr>
<tr>
<td>2 Eicosapentaenoic (20:5n3)</td>
<td>76</td>
<td>2nd 40%</td>
<td>19 - 352</td>
</tr>
<tr>
<td>3 Docosapentaenoic (22:5n3)</td>
<td>55</td>
<td>3rd 60%</td>
<td>31 - 112</td>
</tr>
<tr>
<td>4 Docosahexaenoic (22:6n3)</td>
<td>285</td>
<td>4th 80%</td>
<td>95 - 333</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Polyunsaturated Omega-6</strong></th>
<th>Results (µM)</th>
<th>Percentile Ranking by Quintile</th>
<th>95% Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 Linoleic (18:2n6)</td>
<td>1632</td>
<td>1st 20%</td>
<td>1,305 - 3,300</td>
</tr>
<tr>
<td>6 Gamma Linolenic (18:3n6)</td>
<td>10.9</td>
<td>2nd 40%</td>
<td>5.2 - 58.0</td>
</tr>
<tr>
<td>7 Eicosadienoic (20:2n6)</td>
<td>13</td>
<td>3rd 60%</td>
<td>14 - 45</td>
</tr>
<tr>
<td>8 Dihomogamma Linolenic (20:3n6)</td>
<td>98</td>
<td>4th 80%</td>
<td>64 - 294</td>
</tr>
<tr>
<td>9 Arachidonic (20:4n6)</td>
<td>530</td>
<td>1st 20%</td>
<td>260 - 760</td>
</tr>
<tr>
<td>10 Docosadienoic (22:2n6)</td>
<td>1.1</td>
<td>2nd 40%</td>
<td>0.0 - 3.8</td>
</tr>
<tr>
<td>11 Docosaeicosenoic (22:4n6)</td>
<td>13</td>
<td>3rd 60%</td>
<td>7 - 51</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Polyunsaturated Omega-9</strong></th>
<th>Results (µM)</th>
<th>Percentile Ranking by Quintile</th>
<th>95% Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 Mead (20:3n9)</td>
<td>3.0</td>
<td>1st 20%</td>
<td>0.5 - 13.2</td>
</tr>
</tbody>
</table>

### Monounsaturated

<table>
<thead>
<tr>
<th><strong>Polyunsaturated</strong></th>
<th>Results (µM)</th>
<th>Percentile Ranking by Quintile</th>
<th>95% Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 Myristoleic (14:1n5)</td>
<td>1.9</td>
<td>1st 20%</td>
<td>&lt;= 11.6</td>
</tr>
<tr>
<td>14 Palmitoleic (16:1n7)</td>
<td>54</td>
<td>2nd 40%</td>
<td>&lt;= 235</td>
</tr>
<tr>
<td>15 Vaccoleoic (18:1n7)</td>
<td>94</td>
<td>3rd 60%</td>
<td>74 - 209</td>
</tr>
<tr>
<td>16 Oleic (18:1n9)</td>
<td>1,659</td>
<td>4th 80%</td>
<td>1,079 - 2,800</td>
</tr>
<tr>
<td>17 11-Elcosenoic (20:1n9)</td>
<td>9.4</td>
<td>1st 20%</td>
<td>8.4 - 29.5</td>
</tr>
<tr>
<td>18 Erucic (22:1n9)</td>
<td>3.8</td>
<td>2nd 40%</td>
<td>2.8 - 8.1</td>
</tr>
<tr>
<td>19 Nervonic (24:1n9)</td>
<td>113</td>
<td>3rd 60%</td>
<td>70 - 189</td>
</tr>
</tbody>
</table>
### Fatty Acids - Plasma

Ranges are for ages 13 and over.

#### Percentile Ranking by Quintile

<table>
<thead>
<tr>
<th>Results (μM)</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>Reference Interval</th>
</tr>
</thead>
</table>

#### Saturated

<table>
<thead>
<tr>
<th></th>
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<th>3rd</th>
<th>4th</th>
<th>5th</th>
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</thead>
<tbody>
<tr>
<td>Capric (10:0)</td>
<td>2.1</td>
<td>L</td>
<td></td>
<td></td>
<td>6.6</td>
<td>1.5 - 9.8</td>
</tr>
<tr>
<td>Lauric (12:0)</td>
<td>7.0</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td>6.0 - 41.9</td>
</tr>
<tr>
<td>Myristic (14:0)</td>
<td>40</td>
<td>L</td>
<td>1,510</td>
<td>2,945</td>
<td></td>
<td>34 - 180</td>
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<tr>
<td>Palmitic (16:0)</td>
<td>662</td>
<td></td>
<td>1,086</td>
<td></td>
<td></td>
<td>1,364 - 3,525</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>680</td>
<td></td>
<td>1,486</td>
<td></td>
<td></td>
<td>557 - 1,234</td>
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<tr>
<td>Arachidic (20:0)</td>
<td>26</td>
<td></td>
<td></td>
<td>48</td>
<td></td>
<td>22 - 57</td>
</tr>
<tr>
<td>Behenic (22:0)</td>
<td>99</td>
<td></td>
<td>127</td>
<td></td>
<td></td>
<td>53 - 157</td>
</tr>
<tr>
<td>Lignoceric (24:0)</td>
<td>49</td>
<td></td>
<td>104</td>
<td></td>
<td></td>
<td>42 - 130</td>
</tr>
<tr>
<td>Hexacosanoic (26:0)</td>
<td>&lt;0.5</td>
<td></td>
<td>0.82</td>
<td></td>
<td></td>
<td>&lt;= 0.81</td>
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#### Odd Chain

<table>
<thead>
<tr>
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<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentadecanoic (15:0)</td>
<td>11</td>
<td></td>
<td>29</td>
<td></td>
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<td>&lt;= 35</td>
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<tr>
<td>Heptadecanoic (17:0)</td>
<td>31</td>
<td></td>
<td>45</td>
<td></td>
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<td>&lt;= 53</td>
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<tr>
<td>Nonadecanoic (19:0)</td>
<td>2.8</td>
<td></td>
<td>5.4</td>
<td></td>
<td></td>
<td>&lt;= 6.8</td>
</tr>
<tr>
<td>Heneicosanoic (21:0)</td>
<td>5.0</td>
<td></td>
<td>13.26</td>
<td></td>
<td></td>
<td>&lt;= 17.6</td>
</tr>
<tr>
<td>Tricosanoic (23:0)</td>
<td>21</td>
<td></td>
<td>50</td>
<td></td>
<td></td>
<td>&lt;= 65</td>
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#### Trans

<table>
<thead>
<tr>
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<th>4th</th>
<th>5th</th>
<th></th>
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<tbody>
<tr>
<td>Palmitelaidic (16:1n7t)</td>
<td>1.0</td>
<td></td>
<td>3.0</td>
<td></td>
<td>101</td>
<td>&lt;= 5.2</td>
</tr>
<tr>
<td>Total C:18 Trans</td>
<td>33</td>
<td></td>
<td>101</td>
<td></td>
<td></td>
<td>&lt;= 154</td>
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#### Ratios

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<th>3rd</th>
<th>4th</th>
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<tbody>
<tr>
<td>LA/DGLA</td>
<td>17</td>
<td>0.26</td>
<td>20</td>
<td></td>
<td></td>
<td>8 - 32</td>
</tr>
<tr>
<td>EPA/DGLA</td>
<td>0.78</td>
<td></td>
<td>0.26</td>
<td></td>
<td>11.2</td>
<td>0.12 - 5.23</td>
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<tr>
<td>AA/EPA</td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.9 - 23.7</td>
</tr>
<tr>
<td>Triene/Tetraene</td>
<td>0.006</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;= 0.026</td>
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Fatty Acid Metabolism
(Carnitine & B2)

<p>| | | | |</p>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Adipate</td>
<td>2.8</td>
<td>7.3</td>
</tr>
<tr>
<td>2</td>
<td>Suberate</td>
<td>1.4</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>Ethylmalonate</td>
<td>1.2</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Absorption

<p>| | | |</p>
<table>
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<tbody>
<tr>
<td>LCFAs</td>
<td>1.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Total Fat</td>
<td>1.8</td>
<td>10.1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>22</td>
<td>98</td>
</tr>
</tbody>
</table>

UC** = Unable to Calculate
Omega-3 Index:
Measure of EPA/DHA in RBC

Odds Ratio for Primary Cardiac Arrest: Case Control

90% Reduction in Risk

Total Omega 3 = 5.5
Desired >9% correlates with a 90% risk reduction for sudden cardiac death

USA/UK
Sweden
Japan
Greenland

Deficiency High Risk

Albert et al.
What are the key decision points in using FA in prevention & therapeutics?
Fatty Acid & Phospholipid Therapeutics

Which Molecular Form:

Phospholipid, Triglyceride, or Ethyl Ester?
Molecular Forms of Omega-3’s

**Triglyceride:**
This is the most commonly sold form of fish oils, for which one or more of the fatty acid positions contain EPA or DHA.

**Ethyl Ester:**
Most commonly associated with Omega concentrates where the fatty acids are split from the glycerin backbone to allow it to be more easily concentrated via distillation.

**Phospholipid:**
A phosphate group attached to a glycerol backbone, with two bound fatty acids. Marine phospholipids are unique in that they may contain one or more Omega-3 fatty acids. Phospholipids are a major component of ALL cell membranes.
Marine Phospholipid vs. Soy Phospholipid: Structural & Functional Differences

Linoleic, alpha-linolenic

EPA, DHA

Soy PL

Marine PL

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Fatty Acid & Phospholipid Therapeutics

EPA or DHA, or Both?
Fatty Acid & Phospholipid Therapeutics

What About alpha-Linolenic Acid?
Conversion of alpha-Linolenic acid with age


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Important Dietary Fatty Acids in Cells

- **linoleic acid** 18:2ω-6
- **γ-linolenic acid** GLA 18:3ω-6
- **Dihomo-γ-linolenic acid** DGLA 20:3ω-6
- **arachidonic acid** AA 20:4ω-6
- **docosatetraenoic acid** DTA 22:4ω-6
- **docosapentaenoic acid** DPA 22:5ω-6
- **α-linolenic acid** 18:3ω-3
- **stearidonic acid** 18:4ω-3
- **eicosatetraenoic acid** ETA-3 20:4ω-3
- **eicosapentaenoic acid** EPA-3 20:5ω-3
- **docosapentaenoic acid** DPA 22:5ω-3
- **docosahexaenoic acid** DHA 22:6ω-3

**Sources:**
- Sunflower
- Safflower
- Corn
- Sesame
- Red meat
- Dairy
- Eggs
- Fish (warm)
- Squid
- Algae

**Fatty Acids:**
- **α-linolenic acid** 18:3ω-3
- **γ-linolenic acid** GLA 18:3ω-6
- **stearidonic acid** 18:4ω-3
- **eicosatetraenoic acid** ETA-3 20:4ω-3
- **eicosapentaenoic acid** EPA-3 20:5ω-3
- **docosapentaenoic acid** DPA 22:5ω-3
- **docosahexaenoic acid** DHA 22:6ω-3

**Enzymes:**
- **Δ6 desaturase**
- **Δ5 desaturase**
- **Δ4 desaturase**
- **Elongase**

**Prostanoids:**
- pgd₂, pge₂, pgf₂α, pgl₂, txa₂, lta₄, ltb₄, ltc₄, lte₄
- pgd₃, pge₃, pgf₃α, pgl₃, txa₃, lta₅, ltb₅, ltc₅, lte₅

**Other:**
- A/J-Ring Neuroprostane 17S Resolvins blocks prostanoids

**Fats:**
- Sunflower
- Safflower
- Corn
- Sesame

**Vegetables:**
- Red meat
- Dairy
- Eggs
- Fish (warm)
- Squid
- Algae
- Sunflower
- Safflower
- Corn
- Sesame
- Flax
- Chia
- Pumpkin

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Fatty Acid & Phospholipid Therapeutics

Neural Architecture vs. Neurovascular Effects?
Fatty Acid & Phospholipid Therapeutics

How Important is the n-6:n-3 Ratio?
Omega-6:Omega-3 Ratios in Selected Cases

- TPN Formula: 115
- Revised TPN: 6
- Ideal: 4
- Modern Diet: 30
- Breastmilk (some): 60

DEFINES Human Structure/Function

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### N-6:N-3 Ratio:

**CHD & Fatal Ischemic HD**

<table>
<thead>
<tr>
<th>Fatty Acid Levels * (Lower Risk Category) [based on total % of FA being present as...]</th>
<th>Omega-6:Omega-3</th>
<th>AA:EPA</th>
<th>AA:DHA</th>
<th>AA:(EPA+DHA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Omega-3 (≥7.2)</td>
<td>&lt;4.5</td>
<td>&lt;5.0</td>
<td>&lt;1.8</td>
<td>&lt;1.4</td>
</tr>
<tr>
<td>DHA (≥4.5)</td>
<td>&lt;3.0</td>
<td>&lt;1.1</td>
<td>&lt;1.5</td>
<td>&lt;0.45</td>
</tr>
<tr>
<td>EPA+DHA (≥4.6)</td>
<td>&lt;5.8</td>
<td>&lt;9.2</td>
<td>&lt;2.5</td>
<td>&lt;2.1</td>
</tr>
</tbody>
</table>


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N-6:N-3 Ratio:
CHD & Fatal Ischemic HD

Cut-off Values for the Fatty Acid Ratios that place the Levels of Total Omega-3 Fatty Acids, DHA, and EPA+DHA in the 'Lower Risk' Categories with 95% Confidence Level (2,053 patients)

<table>
<thead>
<tr>
<th>Fatty Acid Levels * (Lower Risk Category)</th>
<th>Omega-6:Omega-3</th>
<th>AA:EPA</th>
<th>AA:DHA</th>
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</tr>
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<tbody>
<tr>
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<td>&lt;4.5</td>
<td>&lt;5.0</td>
<td>&lt;1.8</td>
<td>&lt;1.4</td>
</tr>
<tr>
<td>[total omega-3 &gt;7.2% of total FA]</td>
<td>8.1=US</td>
<td>16.2=US</td>
<td>4.2=US</td>
<td>3.3=US</td>
</tr>
<tr>
<td></td>
<td>2.3=Jpn</td>
<td>1.7=Jpn</td>
<td>0.9=Jpn</td>
<td>0.6=Jpn</td>
</tr>
</tbody>
</table>

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**N-6:N-3 Ratio:**

**What Level for Correction?**

Daily supplementation of North American males with **630 mg EPA** plus **640 mg DHA** for 21 days lowered their ratios into or towards the 'lower risk' categories with newly modified ratios of 3.8, 5.1, 2.1, and 1.5, respectively (Does not address Brain)

<table>
<thead>
<tr>
<th>Fatty Acid Levels * (Lower Risk Category)</th>
<th>Omega-6:Omega-3</th>
<th>AA:EPA</th>
<th>AA:DHA</th>
<th>AA:(EPA+DHA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Omega-3</td>
<td>&lt;3.8</td>
<td>&lt;5.1</td>
<td>&lt;2.1</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td></td>
<td>8.1=US</td>
<td>16.2=US</td>
<td>4.2=US</td>
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</tr>
</tbody>
</table>

**N-6:N-3 Ratio:**

**Effect of Lowering Linoleic Acid**

- Plasma phospholipid fatty acids measured at week 0, 2, 4, 6, and 8,
- LA:ALA Dietary Ratios: 4:0 and 10:1 during the low and high LA diets, respectively.
- Plasma phospholipid LA was higher and EPA was lower during the high than during the low LA diet period ($P < 0.001$),
- DHA declined over the entire 8-wk period ($r = -0.425, P < 0.001$)
- Plasma phospholipid ARA:EPA:
  - $20.7 \pm 1.52$ after high LA diets (4 weeks ($P < 0.001$)
  - $12.9 \pm 1.01$ after high LA diets (4 weeks ($P < 0.001$) LA was inversely associated with EPA ($r = -0.729, P < 0.001$)
- LA was inversely associated with EPA ($r = -0.729, P < 0.001$), but positively associated with ARA:EPA ($r = 0.432, P < 0.001$).
- Conclusion: high LA intakes decrease plasma phospholipid EPA and increase the ARA:EPA ratio

Male Sprague-Dawley rats were fed 1 of 3 diets containing different omega-6/3 PUFA ratios for 2 weeks:

- 5:1
- 1:1
- 1:5

Then, myocardial ischemia was induced by left anterior descending coronary artery occlusion for 40 min, followed by reperfusion.

Cardioprotective mechanisms were studied in the myocardium at 15 min of reperfusion, along with myocardial infarct size after 24 h of reperfusion.

Apoptosis was evaluated in the hippocampus and the amygdala.

N-6:N-3 Ratio:

Effect on Cerebral Infarct-SD Rats

• Infarct size was significantly reduced by 32% in groups 1:5 and 1:1 vs. group 5:1.
• Akt activity was higher in groups 1:5 and 1:1 compared with group 5:1.
• Caspase-3 enzymatic activity doubled in area CA1 and the dentate gyrus (DG) in group 5:1 compared with groups 1:1 and 1:5.
• Caspase-8 enzymatic activity was increased in the DG at 24 h, and caspase-9 was enhanced in CA1 at 24 h in group 5:1 vs. groups 1:1 and 1:5.
• These results demonstrate that the increase in the dietary omega-3 PUFA, at the expense of omega-6 PUFA, reduces infarct size and helps to inhibit apoptosis in the limbic system after MI.

Higher Ratio Linked to Inflam Markers

- 374 healthy men and women
- Multi-adjusted regression analyses revealed:
  - plasma n-3 fatty acids were inversely associated with CRP, IL-6 and TNF-α
  - plasma n-6 fatty acids were inversely associated with CRP, IL-6 and fibrinogen
  - monounsaturated fatty acids were inversely associated with CRP and IL-6 (all p-values < 0.05).
  - the n-6/n-3 ratios exhibited the strongest positive correlations with all the markers studied.

Kalogeropoulos, N, et al. Unsaturated fatty acids are inversely associated and n-6/n-3 ratios are positively related to inflammation and coagulation markers in plasma of apparently healthy adults. Clin Chim Acta 2010 Apr 2;411(7-8):584-91
N-6:N-3 Ratio:

Higher Ratio Linked to Inflam Markers

• One unit increase in n-6/n-3 ratio was associated with:
  • 9.6% (p=0.02) higher likelihood of having hsCRP and with 14.0% (p=0.02) higher likelihood of having IL-6 levels in the highest tertiles of their distributions, i.e. >1.89 mg/L and >1.50 mg/dL respectively.
• Concerning n-3 PUFA levels:
  • one unit increase (i.e., 1% of total fatty acids) was associated with 50.0% (p<0.001) lower likelihood of having hsCRP and with 50.0% (p<0.001) lower likelihood of having IL-6 levels in the highest tertiles of their distributions.

Kalogeropoulos, N, et al. Unsaturated fatty acids are inversely associated and n-6/n-3 ratios are positively related to inflammation and coagulation markers in plasma of apparently healthy adults. Clin Chim Acta 2010 Apr 2;411(7-8):584-91
Fatty Acid & Phospholipid Therapeutics

What is the Proper Dose?
## Considerations for Dose

<table>
<thead>
<tr>
<th>Consideration</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention vs. Therapeutic vs. Maintenance</td>
<td>Clinical requires higher dose</td>
</tr>
<tr>
<td>Blood Fatty Acid Status</td>
<td>Esp EPA &amp; DHA, and ratios. Poor status requires higher dose</td>
</tr>
<tr>
<td>Current Dietary Intake</td>
<td>High n-6:n-3 (or AA:EPA) requires diet change &amp; higher dose</td>
</tr>
<tr>
<td>Past Dietary Intake</td>
<td>Long history of poor n-3 intake may mean poor saturation of slow accretion tissues (i.e. brain)</td>
</tr>
<tr>
<td>Low Fat Diets</td>
<td>Many populations with very low overall fat intake (gymnasts, dancers, wrestlers, anorexia, depression, oral health problems, etc)</td>
</tr>
<tr>
<td>Target Tissue</td>
<td>Accretion rates for brain are slower; 6 mo to 2 years estimated need</td>
</tr>
<tr>
<td>Absorption &amp; Digestion</td>
<td>Malabsorption requires 1) higher dose, 2) liposomal forms, 3) attention to GI</td>
</tr>
<tr>
<td>History of Neural Injury</td>
<td>TBI (concussion), stroke, etc</td>
</tr>
</tbody>
</table>

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Safety Considerations of Dose

Norwegian Scientific Committee for Food Safety (VKM) 2011

• Levels up to 6.9 g/day reviewed
• No adverse events encountered

http://english.vkm.no/dav/ojcooohA.pdf; 2011

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Leaf: Bleeding time (min) increased from 6.22 ± 0.2 (SEM) to 7.02 ± 0.2; statistically significant ($p<0.01$), but still within the reference values. These subjects were using aspirin.

http://english.vkm.no/daw/0jmc0oohha.pdf; 2011
High Dose EPA/DHA and Bleeding:

Conclusion

• 20 controlled studies including a total of 4,659 patients did not report any excess bleeding tendency, despite the fact that many patients used anticoagulant.
• Both TAG and ethyl ester formulations were used in the studies.
• The question is still not resolved, since there are studies reporting no significant changes in bleeding time (EPA and DHA 1.8 – 5.4 g/day)
• With one study reporting a significant increase in bleeding time at 6.9 g/day EPA and DHA in CHD patients on anticoagulant medication (though still within normal ranges).
• No significant impact on bleeding time was observed in two other studies in patients on anticoagulation medication using 3.4 and 5.4 g EPA and DHA per day.

http://english.vkm.no/dav/nu7copodha.pdf; 2011
Adverse Event Reports for EPA/DHA as Drug

- **Standard Dose:** 3,600 mg EPA/DHA/day
- **8 clinical trials**
- **As ethyl ester**
- **Taste perversion only Sx to reach significance** \((p=0.015)\)

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td>23</td>
</tr>
<tr>
<td>Blood triglyceride increase</td>
<td>21</td>
</tr>
<tr>
<td>Eructation</td>
<td>20</td>
</tr>
<tr>
<td>Abdominal distension</td>
<td>17</td>
</tr>
<tr>
<td>Rash</td>
<td>17</td>
</tr>
<tr>
<td>Chest pain</td>
<td>16</td>
</tr>
<tr>
<td>Pruritus</td>
<td>15</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>13</td>
</tr>
<tr>
<td>Dizziness</td>
<td>13</td>
</tr>
<tr>
<td>Blood glucose increase</td>
<td>12</td>
</tr>
<tr>
<td>Constipation</td>
<td>12</td>
</tr>
<tr>
<td>Abdominal discomfort</td>
<td>10</td>
</tr>
<tr>
<td>Flatulence</td>
<td>10</td>
</tr>
<tr>
<td>LDL-cholesterol increase</td>
<td>10</td>
</tr>
<tr>
<td>SUM</td>
<td>209</td>
</tr>
</tbody>
</table>

(http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-654_Omacor.cfm),

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• 4 different doses of an ω-3 PUFA supplement on breast adipose tissue fatty acid content over 6 months of treatment.
• 2.52, 5.04, and 7.56 g EPA+DHA/d,
• The larger doses of 2.52, 5.04, and 7.56 g EPA+DHA/d yielded incremental increases of EPA and DHA in breast fat with mean values that were progressively higher but not significantly different.
• Conclusion: ≥ 2.52 g EPA+DHA/d represents the minimum appropriate dose for elevating EPA and DHA contents in the mammary microenvironment.
• Daily doses up to 7.56 g DHA+EPA were well tolerated with excellent compliance (92.9%)

What is a Basic Strategy to Facilitate Metabolic Fitness with FA and PL?
Fatty Acid Markers in Blood

Advanced Considerations - Assessment

- **Plasma, Serum, Whole Blood, or RBC**

1) **EPA + DHA %** - Critical core measure and early CVD risk indicator
   - Target: 8-10% EPA/DHA
2) **AA:EPA ratio** - A measure of "silent" inflammation
   - Target: 5.1
3) **AA:DHA ratio** - A measure of membrane and signaling balance
   - Target: 2.1
4) **LA:GLA Ratio** - Delta-6-desaturase inhibition by decreased Zn or decreased Mg, increased insulin, or other
   - Target: <150
5) **EPA:DGLA Ratio** - Helps balance intake of Series-3 and Series-1 eicosanoid precursors
   - Target: <1
6) **Omega-6:Omega-3 Ratio**
   - Target: 3.8

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Omega-3 & Metabolic Fitness

Supplement & Diet Considerations

- **Brain-fitness-centered fatty acid and phospholipid intake**

1) Long-chain fatty acids (EPA/DHA; ALA)
2) Omega-3-enriched phospholipids, triglycerides (ethyl esters)
   - strongly consider phospholipid forms of omega-3
3) Dose range dependent upon objective
   - 1.0g to 3.00 g/day (Inst of Med)
   - 1.0 g to 5.0 g/day (some sources)
   - 1.0 g to 6.9 g/day (high end of dose range)
4) Target omega-6:Omega-3 ratio between 4:1 and 1:1
5) Limit omega-6 fatty acids in diet
Metabolic Fitness: The Biological Imperative

Complex Tasks + N-3 lipid Loading >>>

- Biological change
- Metabolic Fitness

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Metabolic Fitness: The Biological Imperative

Membrane Lipids

We are building & maintaining -10 football fields of cell membrane area.

These are not simple nutrients.

N-3 lipids an imperative for metabolic fitness.

Increased physical/cognitive load requires increased precursors.

Structural

Signaling

Repair-Resolving

Processing

Brain Fats=26% of net brain ATP.

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Michael A. Schmidt, Ph.D.
MetaboLogics, LLC
Advanced Pattern Analysis and Countermeasures Group
Research Innovation Center
Infectious Disease Research Complex
Colorado State University
3185 Rampart Road
Fort Collins, CO 80521

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