Future directions for nutritional and therapeutic research in omega-3 lipids

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University of Southampton
Aim ...

- To review dietary sources and intakes of long chain ω-3 fatty acids
- To review ω-3 status in human blood and cells and how this may be altered
- To review the impact of ω-3 fatty acids on human health – mechanisms involved - cvd focus
- To highlight strategies to increase long chain ω-3 fatty acid status
- To highlight timely questions
  - EPA vs DHA
  - Plant ω-3 fatty acids (α-linolenic acid & stearidonic acid)
  - Genotype-specific responses
Eicosapentaenoic acid  EPA  20:5\(\omega-3\)

Docosahexaenoic acid  DHA  22:6\(\omega-3\)
Found in seafood, especially oily (fatty) fish, fish oils, liver oils, algal oils ...
## Long chain ω-3 PUFA content of fish (Typical values)

<table>
<thead>
<tr>
<th></th>
<th>EPA (g/100 g food)</th>
<th>DPA (g/100 g food)</th>
<th>DHA (g/100 g food)</th>
<th>Total g/portion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cod</td>
<td>0.08</td>
<td>0.01</td>
<td>0.16</td>
<td>0.30</td>
</tr>
<tr>
<td>Haddock</td>
<td>0.05</td>
<td>0.01</td>
<td>0.10</td>
<td>0.19</td>
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<tr>
<td>Herring</td>
<td>0.51</td>
<td>0.11</td>
<td>0.69</td>
<td>1.56</td>
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<tr>
<td>Mackerel</td>
<td>0.71</td>
<td>0.12</td>
<td>1.10</td>
<td>3.09</td>
</tr>
<tr>
<td>Salmon</td>
<td>0.55</td>
<td>0.14</td>
<td>0.86</td>
<td>1.55</td>
</tr>
<tr>
<td>Crab</td>
<td>0.47</td>
<td>0.08</td>
<td>0.45</td>
<td>0.85</td>
</tr>
<tr>
<td>Prawns</td>
<td>0.06</td>
<td>0.01</td>
<td>0.04</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Typical intakes of long chain $\omega$-3 PUFAs

- Mean UK adult intake is about 0.2 g/day (but bimodal distribution since only 25% of the population consumes oily fish)
- Australian data (Meyer et al. (2003) Lipids 38, 391-398):
  - Mean daily intakes of EPA, DPA and DHA = 0.056, 0.026, and 0.106 g (Total = 0.188 g/d)
  - Median daily intakes of EPA, DPA and DHA = 0.008, 0.006, and 0.015 g DHA (Total = 0.029 g/d)
## Typical contents of EPA and DHA in human blood, cells & tissues (% total fatty acids)

<table>
<thead>
<tr>
<th></th>
<th>EPA</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma PC</td>
<td>0.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Plasma CE</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Plasma TAG</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Platelet PC</td>
<td>0.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Platelet PE</td>
<td>0.6</td>
<td>6.3</td>
</tr>
<tr>
<td>Mononuclear cell PL</td>
<td>0.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Neutrophil PL</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Red cell PL</td>
<td>0.8</td>
<td>3.5</td>
</tr>
<tr>
<td>Brain grey matter PE</td>
<td>-</td>
<td>24.3</td>
</tr>
<tr>
<td>Brain grey matter PS</td>
<td>-</td>
<td>36.6</td>
</tr>
<tr>
<td>Brain grey matter PC</td>
<td>-</td>
<td>3.1</td>
</tr>
<tr>
<td>Brain white matter PE</td>
<td>-</td>
<td>3.4</td>
</tr>
<tr>
<td>Retina PC</td>
<td>-</td>
<td>22.2</td>
</tr>
<tr>
<td>Retina PE</td>
<td>-</td>
<td>18.5</td>
</tr>
<tr>
<td>Retina PS</td>
<td>-</td>
<td>4.6</td>
</tr>
<tr>
<td>Sperm PL</td>
<td>-</td>
<td>35.2</td>
</tr>
<tr>
<td>White adipose tissue</td>
<td>tr</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Time course of incorporation of EPA and DHA into human mononuclear cell phospholipids

Healthy volunteers given fish oil (2.1 g EPA and 1.1 g DHA/day) for 12 weeks

Dose response of incorporation of EPA into human mononuclear cells

Data from Katan et al. (1997) J. Lipid Res. 38, 2012-2022

Serum CE

Red blood cells
Adipose tissue
What is the health impact of increased intake (& status) of long chain ω-3 PUFAS?
Prospective study of ω-3 PUFA intake and CHD outcomes: The Nurse’s Health Study

Prospective study of ω-3 PUFA status and sudden death: The Physician’s Health Study

# CVD: Classic and emerging risk factors

<table>
<thead>
<tr>
<th>CLASSIC:</th>
<th>EMERGING:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>High serum triglycerides</td>
</tr>
<tr>
<td>Gender</td>
<td>Elevated post-prandial lipoaemia</td>
</tr>
<tr>
<td>Family history (genetics)</td>
<td>Endothelial dysfunction</td>
</tr>
<tr>
<td>Smoking</td>
<td>Tendency towards thrombosis</td>
</tr>
<tr>
<td></td>
<td>Inflammation</td>
</tr>
<tr>
<td>High alcohol consumption</td>
<td>Elevated plasma homocysteine</td>
</tr>
<tr>
<td>High blood pressure</td>
<td>Poor antioxidant status</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
</tr>
<tr>
<td>Lack of physical activity</td>
<td></td>
</tr>
<tr>
<td>High serum cholesterol</td>
<td></td>
</tr>
</tbody>
</table>
**CVD: Classic and emerging risk factors**

**CLASSIC:**
- Age
- Gender
- Family history (genetics)
- Smoking
- High alcohol consumption
- High blood pressure
- Diabetes
- Obesity
- Lack of physical activity
- High serum cholesterol

**EMERGING:**
- High serum triglycerides
- Elevated post-prandial lipoaemia
- Endothelial dysfunction
- Tendency towards thrombosis
- Inflammation
- Elevated plasma homocysteine
- Poor antioxidant status

= Improved by ω-3 fatty acids
ω-3 fatty acids most likely slow or limit atherosclerosis due to risk factor reduction ….
but ω-3 fatty acids also reduce risk of coronary events in people with advanced atherosclerosis
GISSI Prevenzione Study

- Patients with MI within the last 3 months assigned to $\omega$-3 fatty acids (ca. 0.9 g/d) vs. placebo

- Follow up for 3.5 years

- 356 deaths and non-fatal CV events in $\omega$-3 fatty acid group vs. 414 in placebo group


<table>
<thead>
<tr>
<th>RRR in $\omega$-3 fatty acid group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All fatal events</td>
<td>-20%</td>
</tr>
<tr>
<td>CV death</td>
<td>-30%</td>
</tr>
<tr>
<td>Coronary death</td>
<td>-35%</td>
</tr>
<tr>
<td>Sudden death</td>
<td>-45%</td>
</tr>
<tr>
<td>Other deaths</td>
<td>-1%</td>
</tr>
</tbody>
</table>
Marchioli et al. (2002) Circulation 105, 1897-1903
Possible mechanisms for prevention of non-fatal and fatal events with ω-3 fatty acids

1. Decrease cardiac arrhythmias
2. Decrease thrombosis
3. Decrease inflammation
The benefits of long chain ω-3 PUFAs go beyond cardiovascular health
Long chain ω-3 PUFAs are important in:

- membrane structure
- growth
- development and function of brain, neural tissue and eye
- regulation of
  - blood pressure
  - platelet function, thrombosis, fibrinolysis
  - blood lipid concentrations
- vascular function
- cardiac rhythm
- inflammation
- immune response
- bone health
- insulin sensitivity
LC ω-3 PUFAs are protective against:

- hypertension
- hypertriglyceridemia
- thrombosis
- vascular dysfunction
- cardiac arrhythmias
- cardiovascular disease
- inflammatory conditions
- allergic conditions
- immune dysfunction
- insulin resistance
- neurodegenerative diseases of ageing
- bone loss
- some cancers

LC ω-3 PUFAs promote:

- optimal brain growth
- optimal visual and neural function
Increased long chain ω-3 fatty acid supply

→

Altered fatty acid composition of cell membranes

(more EPA & DHA)

→

Improved cell “phenotype”

→

Improved health (real or potential) or clinical outcome

→

Dietary recommendations or new therapeutic potential
There is a clear need to increase intake and status of long chain $\omega-3$ PUFAs
But … in many cases high intakes of $\omega$-3 PUFAS are needed to elicit the desired effects.

Mozaffarian et al. (2006) JAMA 296, 1885-1899
Implications of this for:

- fish – one salmon or mackerel a day?
- supplements – need to deliver > 1 g EPA+DHA (several capsules/day)
- functional foods – how to deliver the desired amounts?

- Used long chain ω-3 PUFA enriched foods (biscuits, bread, cheese spread, chocolate, dips, eggs, margarine, milk, muesli, porridge, cakes, dressing, soups)
- Subjects consumed eight servings of enriched foods/day for 6 months
- Each serving would provide about 0.125 g EPA+DHA
- Mean intake of EPA+DHA increased from 0.2 to 1 g EPA+DHA/d
- EPA and DHA increased in red blood cells
“There was **no significant difference** between omega-3 and control groups in the effects of the intervention for 3 or 6 months on the following parameters: systolic and diastolic blood pressure, compliance of small and large arteries, blood glucose, insulin, lipoprotein lipids (total, HDL and LDL cholesterol and TG), CRP or urinary 11-dehydro-TXB₂.”
Achieving an effective intake (from fish, supplements or functional foods) may be difficult and is an important issue.
There are several other unresolved questions of importance
EPA or DHA?
⇒ EPA and DHA may have different effects and so cannot be regarded as equivalent
What about \( \alpha \)-linolenic acid?
Is $\alpha$-linolenic acid an alternative $\omega$-3 PUFAs?

$\alpha$-Linolenic acid (18:3$\omega$-3)

- STA (18:4$\omega$-3)
- 20:4$\omega$-3
- EPA (20:5$\omega$-3)
- DPA (22:5$\omega$-3) $\rightarrow$ DHA (22:6$\omega$-3)

1. Can $\alpha$-linolenic acid mimic the effects of long chain $\omega$-3 PUFAs?

2. Can $\alpha$-linolenic acid be converted to long chain $\omega$-3 PUFAs in humans?
Finnegan et al. (2003)

Used margarines enriched in $\alpha$LNA or EPA+DHA for 6 months
Functional effects of $\alpha$LNA?

1. Relatively small increases in $\alpha$LNA intake by subjects consuming typical amounts of $\alpha$LNA (to give a total intake < 3.5 g/day) have little, if any, functional effect.

2. Greater increases in $\alpha$LNA intake (to give a total intake > 5 g/day) have some functional effects due to $\alpha$LNA itself or due to conversion to EPA?
Is \(\alpha\)-linolenic acid an adequate precursor for EPA and DHA in humans?
Two approaches have been used:
1. Increase intake of $\alpha$LNA and look at alterations in fatty acid compositions
2. Stable isotope studies to trace $\alpha$LNA metabolism
9.5 g αLNA/day  Six months  Plasma PL

Effect of increasing $\alpha$LNA on EPA and DHA content of plasma PL (human studies)

Estimated conversion efficiency based on kinetic modelling

Conversion efficiency:
- $\alpha$LNA: 0.2%
- EPA: 63%
- DPA: 37%

Overall conversion efficiency:
- 0.13%
- 0.05%

Pawlosky et al. (2001) J. Lipid Res. 42, 1257-1265
Conversion of α-linolenic acid to longer-chain PUFA in men and women

Dietary studies show that αLNA is converted to EPA (and DPA), but not to DHA in humans.

αLNA can mimic some effects of long chain ω-3 PUFAs but at a lower potency (ca. 10%).

Stable isotope studies show that conversion of αLNA to long chain ω-3 PUFAs, especially to DHA, is limited.

But conversion appears to be greater in females than males.

We do not know much about conversion in infants, adolescents, pregnant/lactating women, or the elderly.
\( \Rightarrow \alpha \text{LNA is NOT a replacement for preformed long chain } \omega-3 \text{ PUFAs} \)
What about stearidonic acid?

\begin{align*}
\alpha\text{-Linolenic acid} (18:3\omega-3) & \rightarrow \text{STA} (18:4\omega-3) \\
20:4\omega-3 & \rightarrow \text{EPA} (20:5\omega-3) \\
\text{DPA} (22:5\omega-3) & \rightarrow \text{DHA} (22:6\omega-3)
\end{align*}
0.75 g STA/d 3 weeks then 1.5 g/d 3 weeks
Plasma PL

0.75 g EPA or STA or αLNA/d 3 weeks then 1.5 g/d 3 weeks

Plasma PL

EPA status

Subjects on... EPA STA αLNA

Before

After

⇒ Stearidonic acid produces more EPA than αLNA but is (also) not converted to DHA
### Potential strategies to increase long chain ω-3 PUFA status in humans

<table>
<thead>
<tr>
<th>Metabolic</th>
<th>Dietary</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Provide pre-formed long chain ω-3 PUFAs</strong></td>
<td><strong>Oily fish</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Fish oil capsules</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Fortified or enriched foods</strong></td>
</tr>
<tr>
<td><strong>Provide the precursor α-linolenic acid (18:3ω-3)</strong></td>
<td><strong>Vegetable oils (e.g. soybean, rapeseed)</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Flaxseed/Flaxseed oil</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Fortified or enriched foods</strong></td>
</tr>
<tr>
<td><strong>Provide the precursor stearidonic acid (18:4ω-3)</strong></td>
<td><strong>Unusual vegetable oils</strong></td>
</tr>
</tbody>
</table>
## Potential strategies to increase long chain ω-3 PUFA status in humans

<table>
<thead>
<tr>
<th>Metabolic</th>
<th>Dietary</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provide pre-formed long chain ω-3 PUFAs</td>
<td>Oily fish</td>
<td>Good but may not be viable</td>
</tr>
<tr>
<td></td>
<td>Fish oil capsules</td>
<td>Good but may not be desirable for populations (algal oils)</td>
</tr>
<tr>
<td></td>
<td>Fortified or enriched foods</td>
<td>Good future potential but limited foods currently available</td>
</tr>
<tr>
<td>Provide precursor α-linolenic acid (or STA)</td>
<td>Vegetable oils</td>
<td>May be a viable way to increase EPA but requires high intake</td>
</tr>
<tr>
<td></td>
<td>Flaxseed/Flaxseed oil</td>
<td>Does NOT increase DHA</td>
</tr>
<tr>
<td></td>
<td>Fortified or enriched foods</td>
<td>Need to decrease linoleic acid intake too (competition)</td>
</tr>
</tbody>
</table>
These general statements assume that all individuals, irrespective of gender, age, physiological state, genetics etc. will respond to $\omega$-3 PUFAs in the same way.

This may not be the case.

Certainly we now know that genotype may be important in determining the effect of long chain $\omega$-3 PUFAs.
<table>
<thead>
<tr>
<th>Variable</th>
<th>All (n=50)</th>
<th>ApoE2 (n=8)</th>
<th>ApoE3 (n=22)</th>
<th>ApoE4 (n=20)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>−35.3±5.3†</td>
<td>−30.7±7.6</td>
<td>−34.9±7.0</td>
<td>−37.6±10.6</td>
<td>0.561</td>
</tr>
<tr>
<td>TG AUC</td>
<td>−23.3±3.0†</td>
<td>−32.5±4.6</td>
<td>−18.4±4.3</td>
<td>−24.8±5.2</td>
<td>0.136</td>
</tr>
<tr>
<td>TG IAUC</td>
<td>−7.9±5.6†</td>
<td>−27.7±7.0a</td>
<td>−2.7±10.0b</td>
<td>−5.5±8.2b</td>
<td>0.023</td>
</tr>
<tr>
<td>TC</td>
<td>−1.5±2.1</td>
<td>−1.1±3.8a</td>
<td>−6.3±2.8ab</td>
<td>3.5±3.5abc</td>
<td>0.014</td>
</tr>
<tr>
<td>HDL-C</td>
<td>−0.8±3.3</td>
<td>12.2±5.5</td>
<td>0.6±4.1</td>
<td>−7.4±6.3</td>
<td>0.806</td>
</tr>
<tr>
<td>LDL-C</td>
<td>7.1±3.2‡</td>
<td>3.1±5.4</td>
<td>0.6±4.5</td>
<td>15.9±4.0</td>
<td>0.120</td>
</tr>
<tr>
<td>% LDL-3</td>
<td>−26.0±8.3†</td>
<td>−31.0±40.3a</td>
<td>−17.2±8.8ab</td>
<td>−35.7±13.6abc</td>
<td>0.021</td>
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<tr>
<td>LPL</td>
<td>14.7±14.5§</td>
<td>47.2±29.7</td>
<td>2.1±9.7</td>
<td>17.3±33.9</td>
<td>0.177</td>
</tr>
<tr>
<td>NEFA</td>
<td>−7.8±4.8†</td>
<td>−22.1±5.9</td>
<td>−5.7±5.7</td>
<td>−4.1±9.9</td>
<td>0.616</td>
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<tr>
<td>NEFA AUC</td>
<td>−7.4±3.2†</td>
<td>−18.3±6.7</td>
<td>−4.8±4.7</td>
<td>−5.7±6.3</td>
<td>0.608</td>
</tr>
</tbody>
</table>

Conclusions

- Intake of EPA and DHA is typically low
- Status of EPA and DHA is increased with increased intake (time, dose & pool dependent)
- Increased EPA and DHA intake leads to altered physiology and is associated with improved health
- But effects may require > 0.75 g/day
- EPA and DHA probably have different but overlapping effects
- $\alpha$-LNA increases EPA but not DHA status
- $\alpha$-LNA is less potent than EPA
- SDA increase EPA more than $\alpha$-LNA does
- There are individual-specific responses to EPA and DHA that depend upon genotype -> a challenge and an opportunity