Long-chain Omega-3 Oils: Sources, Ingredient quality and Methods of Analysis

Peter D Nichols

NZIFST, June 30, 2015
**LC Omega-3 Oils: Coverage today**

- **Health benefits** – 1967-2013: 3100 Clinical Studies & 28,300 Research Articles on Omega-3
- **Shorter chain versus Long-chain** – Consumer confusion
- **Farmed seafood** – An update on *LC Omega-3* oil trends
- **Supplements** – Selected Australian products, including Fish, Calamari & Krill oils
- **Sources of *LC Omega-3*** – Future sources, an Australian perspective; new *land plant* LC-Omega-3
- **Analytical methods issues** – Observations & feedback with *LC Omega-3* oils in Australia & NZ
- **Summary**
Essential Fatty Acid Families

**ω6 family**

18:2ω6  
Linoleic (LA)

Corn Oil  
Safflower Oil  
Sunflower Oil

20:4ω6  
Arachidonic AA

Meat, Eggs, Brains

**ω3 family**

18:3ω3  
α-Linolenic (ALA)

Flaxseed Oil  
Canola Oil  
Soybean Oil

H3C

20:5ω3  
Eicosapentaenoic EPA

H3C

22:6ω3  
Docosahexaenoic DHA

Microalgae  
Seafood

**Thrombotic**  
**Inflammatory**

**Anti-thrombotic**  
**Anti-inflammatory**

LC Omega-3 Oils: ≥C20, two or more double bonds
Health benefits from LC Omega-3 Oils

- Prevention of CHD
- Lower blood pressure and plasma / serum lipid levels
- Anti-thrombotic
- Anti-inflammatory
- Neuropsychiatric disorders, including Alzheimers
- ADHD / related disorders
- Obesity / weight loss
- Asthma
- Muscle growth enhanced
- Kidney & liver disorders
- Spinal chord repair

Infant nutrition
- Brain & retina development

Aquaculture & animal feed
- Larval nutrition
- Product quality
**Shorter chain v Long-chain Omega-3**

Critical Reviews in Food Science and Nutrition

Jumping on the Omega-3 Bandwagon: Distinguishing the Role of Long-Chain and Short-Chain Omega-3 Fatty Acids

Giovanni M. Turchini et al. (2012)

- **LC Omega-3** are almost unanimously recognized for their health benefits.

- Only limited evidence of any health benefit is currently available specifically for the main precursor of these fatty acids – **ALA**.

- **ALA & LC Omega-3** commonly referred to as “omega-3” fatty acids. It is difficult for consumers to recognize this difference.

- Food labelling legislations allow products containing ALA & without LC Omega-3 to be marketed as “omega-3 source”; this may confuse consumers in choosing more healthy diets.

- A case for regulatory bodies was proposed to clearly distinguish food products containing only ALA from foods containing LC Omega-3. Such information could empower consumers to make more informed choices.
Shorter chain v Long-chain Omega-3

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- ALA & LC Omega-3 commonly referred to as “omega-3” fatty acids. It is difficult for consumers to recognize this difference.
- Food labelling legislations allow products containing ALA & without LC Omega-3 to be marketed as “omega-3 source”; this may confuse consumers in choosing more healthy diets.
- A case for regulatory bodies was proposed to clearly distinguish food products containing only ALA from foods containing LC Omega-3. Such information could empower consumers to make more informed choices.
How Much *LC Omega-3* Do We need?

Average intake - *EPA + DHA* in Australia:
- 30 mg/day (Bureau of Statistics 1995);
- 175 mg/day (Howe et al. 2006)

2006 – NHMRC: Suggested Dietary Targets:
- 610 mg/day - men, 430 mg/day - women
- 1000-2000 mg/day for CHD patients

- Most fish oil supplements - 18% *EPA* + 12% *DHA*
  2 g fish oil supplies the *SDT* for *EPA + DHA*

- Two meals of oily fish (e.g. farmed salmon)
Aquaculture - Has the Good Oil gone missing?

July 2002, INFORM, AOCS:

“warned that some species of farm-raised fish may have little or no omega-3 fatty acids……”

(Stoll, Harvard Med. School)

Farm fish fail omega-3 trial

Emma Ross

THE health benefit of eating oily fish such as salmon and mackerel seems to depend on what the fish are fed, new research has revealed. Also, adding vegetable oil to feed pellets appears to dilute the powerful heart disease fighting effect.

Fatty fish such as salmon, tuna, mackerel, sardines and herring are rich in omega-3 fatty acids, the healthy fat that scientists believe raises the good HDL cholesterol, lowers unhealthy triglycerides and slows the growth of plaque, protecting the heart from disease.

However, in modern fish farming, the fish are usually fed pellets that contain a mixture of natural fish oil and vegetable oil.

In a study presented at the annual meeting of the European Society of Cardiology, Norwegian scientists showed that people who ate salmon fed on pure vegetable oil, or on 50 per cent fish oil and 50 per cent vegetable oil, did not get any meaningful improvement in the relevant blood tests.

The research involved 38 people with heart disease in Oslo, Norway. The fish was farmed in northwest Norway, colour-coded according to the pellets they were fed and shipped to a central kitchen in Oslo where they were transformed into meals and served.

One-third of the volunteers were fed salmon that had been given pellets of fish oil, another third got fish fed on a 50-50 mix of fish oil and rapeseed oil, and the last group got salmon reared on pure rapeseed oil pellets. Each volunteer ate 50g of the fish per week, or one fish meal per day, for six weeks.

The scientists, led by Dr Harold Arnessen of Ullevaal University Hospital in Norway, examined the blood of the volunteers at the beginning and the end of the six-week period to see the concentrations of omega-3 fatty acids and track changes in blood chemicals linked to heart disease.

“The composition of the food pellets was mirrored in the flesh of the salmon fillets and again mirrored in the serum fatty acids of the patients,” Arnessen said.

Omega-3 levels increased substantially in the patients who ate salmon fed on fish oil, but not in the patients who ate salmon fed on mixed pellets or vegetable oil pellets. The results were the same for improvements in chemical markers of inflammation, which is involved in building plaque in the arteries.

The most impressive difference was in triglycerides, which fell by 30 per cent in the fish oil group and not at all in the patients who ate fish reared on the vegetable oil or the fish oil mix. Triglycerides are the chemical form in which most fat exists in food and in the body.

Everybody’s cholesterol dropped, but that was probably because they were eating fish instead of meat, which is high in saturated fat, the scientists concluded. Nobody lost weight during the study, which means the results could not have been due to differences in weight loss, Arnessen said.

“Only two percent of the market today is wild salmon. The farmed salmon market today is very close to 90-95 percent. It’s what we have in Norway and it’s more or less the same all over the world,” Arnessen said. “The findings underline the importance of tailoring the salmon with heart protective properties.”

AP
Australian Farmed Fish – Good Oil (2002)

Fish - farmed

<table>
<thead>
<tr>
<th>Fish</th>
<th>LC Omega-3 Oils (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murray cod</td>
<td>100</td>
</tr>
<tr>
<td>silver perch</td>
<td>1000</td>
</tr>
<tr>
<td>striped perch</td>
<td>2500</td>
</tr>
<tr>
<td>barramundi</td>
<td>2000</td>
</tr>
<tr>
<td>Atlantic salmon</td>
<td>2000</td>
</tr>
</tbody>
</table>

Average - Wild fish

Nichols et al. 2002
Australian Farmed Fish – Good Oil (2002-14)

Global fish catches static or declining
• Fish oil used in aquaculture – replaced by other oils

LC Omega-3 oils have decreased cf 2002

• Content decreased by 10-50+% in 2010-14

• ω3/ω6 ratio <1 in 2012-14

Farmed Atlantic salmon – LC Omega-3 Content

Nichols et al. Nutrients 2014 - (2002-2013 results)
Farmed Atlantic salmon - %LA, %EPA, %DHA

Changing diets:
- % LA increasing
- % EPA+DHA decreasing

Nichols et al. Nutrients 2014 - (2002-2013 results)
Farmed Atlantic salmon – Omega-3 / Omega-6 ratio

- Ratio decreasing with increased use of (Chicken Fat) in feed
- Ratio less than 1 since mid 2013

Nichols et al. Nutrients 2014 – (2002-2013 results)
Comparison of farmed Tasmanian & NZ salmon: 
**EPA+DHA** content (mg/100 g)

NZ King salmon data – c/o Matt Miller, Plant & Food Res.

Nichols et al. Nutrients 2014
Fish, Squid & Krill Oil Capsules

Three Product Groups
Cost per annum – 500 mg/day

18/12 oils $20-80
Enriched oils $90-400
Other oils $130-2000

Nichols et al. Nutrients 2014 - (FO & KO results, SO unpublished data)
Global Fisheries – are there enough fish anyway - ?

We estimate that large predatory fish biomass today is only about 10% of pre-industrial levels.

Loved to death: our fish stocks in crisis
Global Fisheries: are there enough fish anyway – papers II, III, IV……

Worm (2006) Science - “..there would be no fish left by 2048”…

Worm, Hilborn et al. (2009) Science. “ In 5 of 10 well-studied ecosystems, average exploitation rate declined and is now at or below the rate predicted to achieve maximum sustainable yield for 7 systems”…

Ray Hilborn (2010 pers. comm.) “An interesting aspect is that the environmental impact of marine fisheries is seen as much less than for production of animal protein from agriculture that requires removal of forest & in many dimensions is less than for vegetarian diets”

Smith et al. (2011) Science “New approach to sustain ‘forage’ fishing. Reduced catches of small oceanic ‘forage’ fish like sardines & anchovies may be required in some areas to protect larger predators that rely on these species for food.”
Other Sources of LC Omega-3. I.

Microalgae: several University-Industry consortia

- **Phototrophs** (open ponds)
- **Heterotrophs** (fermenters)

- Recent move in algal biofuels R&D towards **HTP** rather than forming **biodiesel (FAME)**
- **LC Omega-3** directed activities
Other Sources of LC Omega-3. II.

Krill: new collaboration of Aker-IMAS at Utas is due to start (ARC-Linkage)

- Resource monitoring & catch limits overseen by CCAMLR (Hobart HQ)
- MSC certified fishery (Aker)
- AAD, IMAS - Stephen Nicol (2014), Assoc Prof:

“The Antarctic krill fishery has been a source of controversy for several years, mainly because of perception that it is not sustainable. However, on several studies and research reports many experts have found the opposite to be true – that the Antarctic krill fishery is actually one of the most sustainably managed in the world.”
Food, Nutrition & Bioproducts Flagship: LC Omega-3 Oils

- LC Omega-3 oils essential for human & marine fish health
- Global fish catches static or declining
- Microalgae biosynthesize the LC omega-3 oils that fish consume & store. Fish do not make EPA+DHA

CSIRO-wide project

Goal: Isolate omega-3 genes from microalgae & transfer them to crop plants to sustainably produce LC omega-3 oils
LC Omega-3 in Marine Microalgae

- **Rhodophyceae**
- **Eustigmatophyceae**
- **Chlorophyceae**
- **Prasinophyceae**
- **Bacillariophyceae**
- **Prymnesiophyceae**
- **Cryptophyceae**
- **Dinophyceae**

**Algal Classes**
LC Omega-3 Oils - engineering in land plants

16:0 → 18:0 → 18:1 → 18:2 → α-18:3

Land Plants

Δ6-des

18:4 SDA

Δ6-elo

20:4

Δ5-des

20:5 EPA

Δ5-elo

22:5

Δ4-des

22:6 DHA

Marine Algae
Land Plant Achievements

First land plant with **EPA + DHA** in its seed oil

```
<table>
<thead>
<tr>
<th></th>
<th>Percent Long-chain Omega-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Land Plants</td>
<td>0%</td>
</tr>
<tr>
<td>CSIRO Model Plant</td>
<td>2.4% EPA</td>
</tr>
<tr>
<td></td>
<td>0.5% DHA</td>
</tr>
</tbody>
</table>
```

*Arabidopsis thaliana*

Robert et al. FPB - 2005
DHA Biosynthesis - Isolation of an efficient synthesis pathway

18:3\(\Delta^9,12,15\)
\(\alpha\)-Linolenic acid, ALA

\textbf{Microalgal}
Petrie et al. 2010; Metab Eng. 12:233-240

18:4\(\Delta^6,9,12,15\)
Stearidonic acid, SDA

\textbf{Microalgal}
Petrie et al. 2010; Marine Biotechnol. 4:430-438

\textbf{Microalgal}
Zhou et al. 2007; Phytochem. 6:785-796

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Zhou et al. 2007; Phytochem. 6:785-796

\textbf{Pyramimonas cordata} \(\Delta^6\)-elo

20:4\(\Delta^8,11,14,17\)
Eicosatetraenoic acid, ETA

\textbf{Pyramimonas cordata} \(\Delta^5\)-elo

20:5\(\Delta^5,8,11,14,17\)
Eicosapentaenoic acid, EPA

\textbf{Pyramimonas cordata} \(\Delta^5\)-elo

22:5\(\Delta^7,10,13,16,19\)
Docosapentaenoic acid, DPA

\textbf{Pyramimonas cordata} \(\Delta^4\)-des

22:6\(\Delta^4,7,10,13,16,19\)
Docosahexaenoic acid, DHA
**Pyramimonas Δ5-elongase function in planta (benth leaf)**

- **High Δ5-elongation**
- **Low C20 & C22 intermediates**

*Petrie & Singh 2011 AOB*
2010
Partnership between CSIRO, Nuseed & GRDC
Fish oil-like levels of DHA in plant seed (& high ω3/ω6 ratio)

Benth leaf TAG

Arabidopsis seed

Petrie et al. PLOS ONE 2012; 2014 (Camelina)
# DHA Seed Oil: *Arabidopsis T₄ Profile*

<table>
<thead>
<tr>
<th></th>
<th>Columbia (weight % of TFA)</th>
<th>T₄Col_22.2 (weight % of TFA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA</td>
<td>12.5%</td>
<td>3.3%</td>
</tr>
<tr>
<td>LA</td>
<td>29.1%</td>
<td>4.3%</td>
</tr>
<tr>
<td>ALA</td>
<td>18.3%</td>
<td>29.5%</td>
</tr>
<tr>
<td>GLA</td>
<td></td>
<td>0.4%</td>
</tr>
<tr>
<td>DGLA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:4ω6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPA6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDA</td>
<td></td>
<td>5.5%</td>
</tr>
<tr>
<td>ETA</td>
<td></td>
<td>0.8%</td>
</tr>
<tr>
<td>EPA</td>
<td></td>
<td>1.8%</td>
</tr>
<tr>
<td>DPA</td>
<td></td>
<td>1.5%</td>
</tr>
<tr>
<td>DHA</td>
<td></td>
<td>15.1%</td>
</tr>
</tbody>
</table>

Similar Results for:
- *Camelina*
- *Canola*

New Omega-3

New LC Omega-3

Petrie et al. PLOS ONE (2012)
History of DHA biosynthesis attempts

Petrie et al. PLOS ONE 2014; Inform 2013
CSIRO Consumer Research

Trials – Australia, USA, Europe, Asia

Take home message:

A large proportion of the population are accepting of GM land plant LC omega-3 oil that:

• Provides a health benefit,
• Was supported by health claims from a trusted source,
• Was indirectly consumed (e.g. food for farming fish)

(Cox et al, 2008, 2010)
Other (New) Sources of LC Omega-3. III.

**DHA synthesis in oil seeds: application to aquafeeds**

- Petrie et al. Inform 2013, PLOS ONE 2014

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Engineered oilseed crops with fish oil DHA levels

James R. Petrie, Peter D. Nichols, Mustafa Devleen, and Sudinder R. Singh

The aquaculture industry has been a major user of fish oil for several decades. Owing to increasing concern over the sustainability of this practice, there is a growing interest in investigating alternative sources of long-chain fatty acids, with microalgae being a promising option. However, the practical application of this approach has been limited due to the high cost of extraction and processing.

Consequently, the development of genetically modified (GM) oilseed crops with high levels of DHA has been a focus of research. This approach offers several advantages, including the potential for large-scale production and reduced costs compared to microalgae production. Several studies have reported the successful expression of DHA in various oilseed crops, such as canola, rapeseed, and soybean.

This article provides a progress update on the most challenging long-chain fatty acid genetically engineered oil (GEO).
Current Approaches & Issues with Analysing Long-chain Omega-3: Methods Observations

Use of a range of protocols – all steps

- Extraction
- Methylation
- Use of – standard methods, standards and/or a ‘standard reference material’
- Derivitization
- GC column
- GC program
- GC drop-off
- Use of GC standards – all long-chain omega-3 FA
- GC-MS verification or NOT
- Reporting - (i) % of TFA vs (ii) mg/100 g (need Int. Std.)
- Methods & data scrutiny / technical & scientific expertise
Reported results – Non-validated

• Long-chain fatty alcohols derived from WE co-elute with EPA & DHA on some polar GC columns

• NUTTAB 2006 - Land plants do not contain EPA & DHA

• NUTTAB 2006 - Meat products – high levels of LC Omega-3 needed further validation
## Analytical Report – Review Process

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Fish A</th>
<th>Fish B</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2</td>
<td>11.1</td>
<td>4.7</td>
</tr>
<tr>
<td>18:2 trans</td>
<td>&lt; 0.1</td>
<td>4.3</td>
</tr>
<tr>
<td>18:3</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>18:3w3</td>
<td>2.6</td>
<td>2.3</td>
</tr>
<tr>
<td>20:0</td>
<td>0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>20:1</td>
<td>1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>20:2</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>20:3 (20:4)</td>
<td>0.9</td>
<td>2.8</td>
</tr>
<tr>
<td>22:4 (22:0)</td>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>24:0</td>
<td>1.8</td>
<td>0.8</td>
</tr>
<tr>
<td>20:5w3</td>
<td>4.9</td>
<td>2.0</td>
</tr>
<tr>
<td>22:6w3</td>
<td>5.5</td>
<td>6.3</td>
</tr>
</tbody>
</table>

*Other PUFA – not reported: 22:5w3 (DPA), 22:5w6, 22:4w6, 20:3w6, ...*
Most FA identifications incorrect

Marine samples
- all low in EPA & DHA
- very high 24:1 & 20:3
- most FA after 18:1 mis-identified
**NZ Fish oils study – Oil Quality I.**

- NZ paper *Nature Scientific Reports* (Albert et al., Jan 2015)

  Two issues reported:
  
  (i) **EPA+DHA did not meet label claim**, markedly so for many (69%) products
  
  (ii) **Oils highly oxidized** (high PV, AnV) – health implications raised, including in media. Although regards health implications, the NZ study authors stated:

    "One relatively short study has compared the effects of oxidized and unoxidized fish oil in humans (32), observing no evidence of acute oxidative stability."

- Considerable negative media

- **O3C, GOED, AAOCS, NZ Oils, TGA** followed up; brief summary:
  
  - Methods issues raised by these groups & by a large range of scientists
  - **O3C** contacted main manufacturers; retesting occurred (see over)
  - **GOED & TGA** have overseen further analyses of Aust & NZ products
    
    - recent update: very different results

- Industry very concerned; considerable feedback to **O3C**
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NZ Fish oils study – Oil Quality II.

Previous studies
• 2014 *Nutrients*, Bengtson Nash et al.
• 2014 *Nutrients*, Nichols et al.
• Several others

Recent analyses since the NZ fish oils study
• Aust & NZ manufacturers - retesting
• GOED – 4 independent labs commissioned, 47 NZ samples
• TGA – 15 Aust & NZ samples

For all publications/sets of analyses above
• Aust & NZ oils generally - *met LC Omega-3 claims & were NOT oxidized*
• Next steps – under discussion
NZ Fish oils study – Oil Quality II.

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Other Negative Media re LC Omega-3

General:
- Retracted by Journal – November 2014

Prostate findings:
- Brasky et al. 2013 JNCI; resultant negative media
- Published responses in same journal, Omega-3 Centre (O3C) response & website materials (Science Advisors - key role): [http://omega-3centre.com/](http://omega-3centre.com/)

Cardiovascular:
- Inuit data revisited, Meta analysis reviews; resultant negative media
- O3C response & website materials (Science Advisors - key role)
Positive News re LC Omega-3

General:

Joint Omega-3 Symposium O3C-AAOCS, Newcastle, November 2013: Published in Nutrients Special Issue (2014). Book also published in late 2014.

“Recent Advances in Omega-3: Health Benefits, Sources, Products and Bioavailability”. See:

http://www.mdpi.com/journal/nutrients/special_issues/omega-3_conference

- 12 papers in the Special Issue. Australian / NZ emphasis
Summary & Future Directions

• Seafood remains an excellent source of **LC Omega-3** for consumers

• **Farmed seafood** in Australia & NZ generally contains higher **LC Omega-3** content than wild harvest seafood

• **LC Omega-3** content in **farmed fish** has decreased, as has the previously high **omega-3 / omega-6** ratio; scope to revisit.

• Alternate sources of **LC Omega-3** are required for future aquaculture application
  - Excellent progress with new land plants (Canola-DHA, CSIRO-Nuseed-GRDC); first field trial harvested in December 2014

• 1 Ha of canola-DHA at 12% DHA = DHA from 10,000 fish

• Aust & NZ fish oil supplements – generally do meet **LC Omega-3 Claims** & are NOT oxidized
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Thank you

Much is underway....

The CSIRO-Nuseed LC Omega-3 Team