New Zealand research – new omega-3 supplement – Lester’s oil

Lynnette R. Ferguson¹, Stephanie Ellett¹, Amalini Jesuthasan¹, Gareth Marlow¹, Shuotun Zhu¹, Mike Agnew², Jeffrey M Greenwood³ and Bobbi Laing¹,
¹ Discipline of Nutrition, Faculty of Medical & Health Science, The University of Auckland, New Zealand and ²AgResearch Ltd, ³New Zealand Institute for Plant & Food Research Limited, Mt. Albert Research Centre, New Zealand
Position statement

Fish, fish oils, n-3 polyunsaturated fatty acids and cardiovascular health

Updated November 2008

Governments

The Heart Foundation encourages governments to:

1. Recommend that Australians consume fish and fish oils.

2. Commit to collecting data on the Australian population’s dietary intake (including of n-3 PUFA) through a regular national nutrition survey.

3. Include fish oil capsules and liquid in the Pharmaceutical Benefits Scheme to help health professionals to prescribe them to people who have CHD.

4. Support professional development for doctors that includes advice on fish and fish oil consumption for those with, and at risk of, CHD.

5. Support sustainable fishing practices and healthy, sustainable marine ecosystems.

6. Monitor levels of methylmercury and dioxins in Australian fish.
Why study (another) omega-3-based dietary supplement?

- Diet is a key component in the disease susceptibility of individuals.
- There is no question that the nature and balance of serum lipids play an important role in health.
- However, there has been some local controversy over the use of dietary supplements containing omega-3 polyunsaturated fatty acids.
Analysis finds most brands contain only 68% of fatty acid amounts listed on label

Consumers sold short on omega-3 oil

7:46 AM Thursday Jan 22, 2015

Fish oil capsules are one of the most popular omega-3 fatty acid supplements. Picture / Getty Images
Nearly all fish oil supplements marketed in New Zealand contain much less of the brain-boosting omega-3 fatty acids than their labels claim, an eye-opening study has found.

When researchers at the University of Auckland's Liggins Institute tested 36 different brands of fish oil capsules, just three contained the same concentrations of omega-3 fatty acids as listed on the label.

Their analysis revealed that the products contained an average of just 68 per cent of the claimed content - and more than two thirds of supplements tested contained less than 67 per cent.

Two products only included only a third of what was on the label.
The research, published in the major journal Nature, also looked at oxidation levels of the products, as omega-3 fatty acids are known to be unstable and can break down when exposed to light, heat and oxygen.

They found that over half had oxidised to a level higher than the recommended limit - and this had nothing to do with the best-before date, price, or country they came from.

The vast amount of oils were sourced from South American deep sea fish, however the researchers deliberately chose not to name the brands and which passed or failed their testing.

When all of the measures the researchers used to evaluate the oxidation levels were applied, only 8 per cent were in line with international recommendations.
Omega-3 fish oils

Docosahexaenoic acid DHA

Eicosapentaenoic acid EPA
Ratios of Fatty Acids in the NZ Diet

% Fatty Acids Current Diet (est)
- Saturated fats
- Trans fats
- Monounsaturated (MUFA)
- n-6 Polyunsaturated (n-6 PUFA)
- n-3 Polyunsaturated (n-3 PUFA)

% Fatty Acids Desirable Diet
NuNZ Lester’s Oil Study

• Omega – 3 fish oil, Vitamin D, Co-Enzyme Q10, Zeaxanthin, Leutin and Astaxanthin

• double blinded, randomised, placebo controlled, cross-over intervention trial, 12 weeks in duration

• 30 healthy participants started and 27 finished.

• Blood, Urine and Faecal samples collected.

• Stool diary and Food Variety Score, Quality of Life
The tested supplement contained a distilled, concentrated, standardised omega-3 ethyl ester with guaranteed levels of EPA and DHA.

**AsureQuality Certificate of analysis**

**GMP**  
Per soft gel  
EPA: 264  
DHA: 185  
Omega 3: 538  
Mercury: <0.01

**NZHM**  
Per soft gel  
EPA: 269  
DHA: 194  
Omega 3: 558  
Mercury: <0.01
<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARSENIC CONTENT</td>
<td>0.0</td>
</tr>
<tr>
<td>CADMIUM CONTENT</td>
<td>0.0</td>
</tr>
<tr>
<td>LEAD CONTENT</td>
<td>0.00</td>
</tr>
<tr>
<td>MERCURY CONTENT</td>
<td>0.00</td>
</tr>
<tr>
<td>ABSORBANCE @ 233nm</td>
<td>0.00</td>
</tr>
<tr>
<td>TOTAL PCB</td>
<td>0.00</td>
</tr>
<tr>
<td>(209 CONGENERS)</td>
<td></td>
</tr>
<tr>
<td>TOTOX VALUE</td>
<td>0</td>
</tr>
<tr>
<td>DIOXIN FURANS</td>
<td>0.00</td>
</tr>
<tr>
<td>WHO-TEQ</td>
<td></td>
</tr>
<tr>
<td>DIOXIN FURANS/DIOXIN LIKE</td>
<td>0</td>
</tr>
<tr>
<td>PCB's WHO-TEQ</td>
<td></td>
</tr>
<tr>
<td>DIOXIN LIKE PCB's</td>
<td>0</td>
</tr>
<tr>
<td>WHO-TEQ</td>
<td></td>
</tr>
<tr>
<td>BENZO (A) PYRENE</td>
<td>0.0</td>
</tr>
</tbody>
</table>
## Specification Limits

<table>
<thead>
<tr>
<th></th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAD - C6:0 CAPROIC</td>
<td>0.0</td>
<td>2.0 %</td>
</tr>
<tr>
<td>FAD - C8:0 CAPRYLIC</td>
<td>50.0</td>
<td>80.0 %</td>
</tr>
<tr>
<td>FAD - C10:0 CAPRIC</td>
<td>20.0</td>
<td>50.0 %</td>
</tr>
<tr>
<td>FAD - C12:0 LAURIC</td>
<td>0.0</td>
<td>3.0 %</td>
</tr>
</tbody>
</table>
Medium chain triglycerides

Hexanoic acid (caproic acid)

Octanoic acid (Caprylic acid)

Decanoic acid (capric acid)

Lauric acid (dodecanoic acid)
Flow Chart for Lester’s Oil Study

Pre-screening, randomisation, n=15 → LO intervention 4 weeks → Washout 4 weeks → Placebo intervention 4 weeks

Pre-screening, randomisation, n=15 → Placebo intervention 4 weeks → Washout 4 weeks → Lester’s oil intervention 4 weeks
Trial design

• Lester’s Oil® supplementation for 4 weeks was compared with an encapsulated medium chain triglyceride (intended as a negative control) on various parameters of disease susceptibility in healthy people (n=30) was recruited from Auckland, New Zealand.

• Various tissue samples (blood, urines stools) were collected before and after each intervention and a range of endpoints including C-reactive protein, plasma lipid metabolic profiling, lipid profiles and lipid peroxidation were taken.
- Lipid Profiling
- Carotenoids
- Ubiquinone
- Vitamin - D

• Proteomics
• DNA Genotyping
• RNA Transcriptomics

• Lipid Peroxidation

• Complete Blood Count

• C Reactive Protein
• Lipid Test
• Polyphenolics

• Targeted/Untargeted metabolomics

• Creatinine

Microbiota
Health to disease continuum
C-reactive protein (CRP) as a measure of inflammation

- Major component of the acute phase response
- <1 mg/mL in normal plasma
- Elevated levels of CRP are indicative of chronic, low-grade inflammation
C-reactive protein differences before and after trials
How Omega-3s Help the Heart:

- By maintaining flexible blood cells and vessels
- By reducing risk of thrombosis
- By reducing blood viscosity
- By reducing blood triglycerides
- By reducing LDL cholesterol
- By increasing HDL cholesterol
- By reducing blood pressure
- By maintaining cardiac rhythm
Role of LDL in Atherosclerosis

LDL Readily Enter the Artery Wall Where They May be Modified

- LDL
- Oxidation of Lipids and ApoB
- Aggregation
- Hydrolysis of Phosphatidylcholine to Lysophosphatidylcholine
- Other Chemical Modifications

Modified LDL

Modified LDL are Proinflammatory

Vessel Lumen

Endothelium

Intima
Role of LDL in atherosclerosis


Modified LDL Promote Differentiation of Monocytes into Macrophages
Role of LDL in Atherosclerosis

Role of LDL in Atherosclerosis

Monocyte
Adhesion Molecules

Vessel Lumen
LDL
Endothelium
MCP-1
Modified LDL
Taken up by Macrohage

Intima
Foam Cell
Macrophage


Lipids Online
Role of LDL in Atherosclerosis

Monocyte → Adhesion Molecules → Cytokines → Macrophage

Vessel Lumen

Remnant Lipoproteins

MCP-1

Modified Remnants

Intima

Foam Cell

Growth Factors
Metalloproteinases
Cell Proliferation
Matrix Degradation

Endothelium

HDL is Protective

Assmann G, ed. *Lipid Metabolism Disorders and Coronary Heart Disease*. Munich: MMV Medizin Verlag, 1993
HDL Prevents Foam Cell Formation


HDL Promotes Cholesterol Efflux

Foam Cell

MCP-1

Modified LDL

LDL

Adhesion Molecules

Cytokines

Macrophage

Vessel Lumen

Endothelium

Intima

Lipids Online
HDL Inhibits Oxidative Modification of LDL

HDL Inhibits Expression of Adhesion Molecules


HDL Inhibits Adhesion Molecule Expression

Vessel Lumen

Endothelium

Modified LDL

HDL Promote Cholesterol Efflux

HDL Inhibit Oxidation of LDL

Intima

Monocyte

Adhesion Molecules

Cytokines

Macrophage

Foam Cell

MCP-1

LDL

Lipids Online
Density

Supplement
Placebo
p=0.0166

Triglycerides in differences before and after Trial
Density

- --- Supplement
- --- Placebo

p=0.0043

HDL in differences before and after Trial
Method for Fatty Acid analysis

• Due to the short time for this trial, changes in fatty acids were considered to be most notable in serum, rather than in red blood cells, which are normally preferred for the ω-3 index.
• The ω-3 index is the sum of EPA and DHA in erythrocyte membranes, expressed as a percentage of total erythrocyte fatty acids.
• This index is used as a risk factor measure for coronary heart disease.
Greatest Protection

- Stavanger\(^1\): > 9.5%: No Added Protection?
- GISSI-P\(^2\): \(\approx\) 9-10%
- CHS\(^3\): 8.8%
- DART\(^4\): \(\approx\) 8-9%
- SCIMO\(^5\): 8.3%
- 5 epi. studies: \(\approx\) 8%
- PHS\(^6\): 7.3%
- Seattle\(^7\): 6.5%

Least Protection

- PHS\(^6\): 3.9%
- SCIMO\(^5\): 3.4%
- Seattle\(^7\): 3.3%

Omega-3 Index Risk Zones

Relative Risk for Death from CHD

- **Undesirable**: 0%
- **Intermediate**: 4%
- **Desirable**: 8% - 10%

Percent of EPA+DHA in RBC

Harris and von Schacky, *Preventive Medicine* 2004
Fatty acid analysis, cont’d

- The samples were collected at the four time points i.e. collected before and after each 4 week intervention.
- The blood was collected and processed and stored until the samples were analysed using the lipid profiling fatty acid methyl esters (FAME) analysis.
- This involves gas chromatography where esterification of lipids and injection, separation, identification and quantitation of the esters occurs.
GLM Lester's Oil

GLM Control

HDL

Triglycerides
Acknowledgements

**Nutrigenomics New Zealand**
Research collaboration between June 2004 and September 2014 among