Effects of a purified, omega-3 rich krill oil phospholipid on cardiovascular disease risk factors and fatty acid composition of erythrocyte membranes in non-human primates

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Presenter: Petter-Arnt Hals MSc PhD
Co-authors: Nils Hoem, Xiaoli Wang, Yong-Fu Xiao
Disclosures

• I am an employee of Aker Biomarine Antarctic AS in Norway. I have no ownership, shares or share options in the company

• The statements made during this presentation do not necessarily represent the view of Aker Biomarine but are merely my own
Topics for the presentation

• Non-human primate study of purified krill oil phospholipids
  – Effects on blood lipids
  – Effect on Omega-3 Index
  – Effects on lipid composition of erythrocyte membranes

• Review of meta-analysis of krill oil studies on blood lipid modification

• Significance of change in Omega-3 Index
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Relevance of animal species in research on treatment of dyslipidemia

Yin et al., J Lipid Res 2012, 53; 51-65
Study design (1)

- Six diabetic, dyslipidemic animals (Cynomolgus) included; 3 controls, 3 treated
- Actives treated with formulated PL98* (PL98 (84%); PEG400 (12.5%); ethanol (3.5%))
- Control substance: water (84%); PEG400 (12.5%); ethanol (3.5%)
- Daily dosing approx. 1 hr after feeding, by gavage

*PL98 is a highly purified and concentrated omega-3 rich phospholipid preparation extracted from krill oil*

**Screening phase**

- Low dose (50 mg/kg) HED ~1g/day
- Intermediate dose (150 mg/kg) HED ~3g/day
- High dose (450 mg/kg) HED ~10g/day
- Post-dose follow-up («Wash-out»)

**Weekly:**
- Blood lipids, apolipoproteins

**Every 2 weeks:**
- BW, diabetes parameters, inflammation markers, LDL particle size, RBC fatty acids incl. Omega-3 Index, endocannabinoids

**Every 4 weeks:**
- Safety parameters, pharmacokinetics

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Hals et al., Lipids Health Dis. 2017, 16; 11
Study design (2)

Primary parameters (weekly)
- Total cholesterol*
- LDL-c*
- HDL-c*
- Non-HDL-c (calculated)*
- ApoB100*
- ApoA1*
- Triglycerides*
- ApoC3
- ApoE
- ApoB48

Secondary parameters (every 2 weeks)
- Diabetes parameters
  - HbA1c; glucose; insulin
- Markers of inflammation
  - CRP; sICAM-1; sVCAM-1; P-selectin; MCP-1
- Omega-3 Index and RBC FA composition*
- LDL particle size*
- Endocannabinoids*
  - AEA, 2-AG, PEA, OEA, DHAEA, EPAEA

Secondary parameters (every 4 weeks)
- Safety parameters
  - BW; AST; ALT; ALP; BUN; Creatinine; UA; Cystatin-C; PT; APTT; TT; WBC and diff’s; RBC; Hct; MCV; MCH; MCHC
- Pharmacokinetics*

*parameters measured bi-weekly during «wash-out»

Hals et al., Lipids Health Dis. 2017, 16; 11
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- Total cholesterol*
- LDL-c*
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*parameters measured bi-weekly during «wash-out»
Effects of PL98 on serum cholesterols in dyslipidemic NHPs

Data presented are control-adjusted mean values

Hals et al., Lipids Health Dis. 2017, 16; 11
Omega-3 Index - Definition

A measure of the amount of EPA+DHA in red blood cell membranes expressed as the percent of total fatty acids

There are 64 fatty acids in this model membrane, 3 of which are EPA or DHA

\[ \frac{3}{64} = 4.6\% \]

Omega-3 Index = 4.6%
Omega-3 Index following administration of PL98 to NHPs

Hals et al., Lipids Health Dis. 2017, 16; 16
EPA and DHA in erythrocytes following administration of PL98 to NHPs

Hals et al., Lipids Health Dis. 2017, 16; 16
Effects of PL98 on fatty acid kinetics in NHP erythrocytes

- Palmitic acid (C16:0)
- Stearic acid (C18:0)
- Dihomo-g-linolenic acid (C20:3n6)
- Oleic acid (C18:1n9)
- Linoleic acid (C18:2n6)
- Docosatetraenoic acid (C22:4n6)
- Arachidonic acid (C20:4n6)
- Docosapentaenoic acid (C22:5n3)

Hals et al., Lipids Health Dis. 2017, 16; 16
Safety parameters for PL98 in dyslipidemic NHPs

Coagulation

- TT
- APTT
- PT

Kidney biomarkers

- CREA
- Uric acid
- BUN

Liver biomarkers

- ALP
- ALT
- AST

Hals et al., Lipids Health Dis. 2017, 16; 11
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• Significance of change in Omega-3 Index
Special Article

Lipid-modifying effects of krill oil in humans: systematic review and meta-analysis of randomized controlled trials

Sorin Ursoniu, Amirhossein Sahebkar, Maria-Corina Serban, Diana Antal, Dimitri P. Mikhailidis, Arrigo Cicero, Vasilios Athyros, Manfredi Rizzo, Jacek Rysz, Maciej Banach; for the Lipid and Blood Pressure Meta-analysis Collaboration Group

• Seven studies with a total of 14 treatment arms reviewed
• A total of 662 subjects – 427 given krill oil, 235 controls
• Both healthy and dyslipidemic subjects included in the studies
• Treatment times between 4 and 12 weeks
• Dose levels between 0.5 and 4 grams/day
Total cholesterol

Original data

Data after Leave-one-out sensitivity analysis

Ursoniu et al., Nutr Rev 2017, 75(5);361-73
Low-density lipoprotein cholesterol

Original data

Data after Leave-one-out sensitivity analysis

Ursoniu et al., Nutr Rev 2017, 75(5);361-73
High-density lipoprotein cholesterol

Original data

Data after Leave-one-out sensitivity analysis
Triglycerides

Original data

Data after Leave-one-out sensitivity analysis
A point to remember re. krill oil: It contains choline!

- Phosphatidyl choline, the main phospholipid in krill oil.

**Choline Deficiency**
- Sensitivity to diet intake modified by estrogen status and genetic variation.
- Decreased methylation capacity, perturbed phosphatidylcholine synthesis, VLDL secretion impaired, PPARα signaling decreased, altered lipid metabolism.

**Liver Disease Progression**
- **Normal Liver**
- **Fatty Liver**
- **Cirrhotic Liver**
- **Carcinoma**

- Mitochondrial dysfunction, reactive oxygen species damage to DNA, epigenetic changes, gene expression altered, ER stress.
- Reactive oxygen species damage to DNA, epigenetic changes, gene expression altered, loss of apoptotic mechanisms.
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  – Effect on Omega-3 index
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• Significance of change in Omega-3 index
Omega-3 Index

A measure of the amount of EPA+DHA in red blood cell membrane phospholipids expressed as the percent of total fatty acids

There are 64 fatty acids in this model membrane, 3 of which are EPA or DHA

\[
\frac{3}{64} = 4.6\%
\]

Omega-3 Index = 4.6%

Harris and von Schacky, Prev Med. 2004, 39; 212-20
The Omega-3 Index is a valid marker of EPA and DHA intake

Relations with Oily Fish Intake ± Fish Oil Capsule Use

Dose response with Fish Oil Capsules (5 months; n=21-24.grp)

Block et al., Open Biomark J. 2008, 1; 1-8

Flock et al., J Am Heart Assoc. 2013, 2; e000513
Human studies indicate a significantly higher increase in Omega-3 Index with phospholipids (krill oil) than with triglycerides (fish oil)

**Study overview**
Single center, double-blinded, placebo-controlled, randomized two-way crossover study

**Subjects**
24 Healthy male and female subjects (12m, 12f)

**Treatment**
- 4-weeks of daily intake
- 8-weeks wash-out between treatments
- 3g krill oil or fish oil, but EPA+DHA was 17% higher in krill oil than in fish oil
- 3g corn oil as placebo

**Results**

**Absolute values (Mean±SD)**

<table>
<thead>
<tr>
<th></th>
<th>Omega-3 Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krill oil</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>Fish oil</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>Corn oil</td>
<td>4.8 ± 0.2</td>
</tr>
</tbody>
</table>

**Mean change, corrected for EPA+DHA dose**

<table>
<thead>
<tr>
<th></th>
<th>Change in Omega-3 Index (% points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krill oil</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>Fish oil</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Corn oil</td>
<td>-0.2 ± 0.1</td>
</tr>
</tbody>
</table>

p<0.0001
The Omega-3 Index correlates with the EPA+DHA content of many tissues (in mice)
In humans the Omega-3 Index correlates well with EPA+DHA levels in the heart

### Study overview

<table>
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<th>Study details</th>
<th>Results</th>
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<td>Heart surgery patients</td>
<td>Correlation between EPA+DHA levels in heart and red blood cells</td>
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**Subjects**
Heart surgery patients

**Study details**
Cardiac and RBC samples were obtained

**Correlation between EPA+DHA levels in heart and red blood cells**

- **Heart EPA + DHA**
- **RBC EPA + DHA**

**Omega-3 Index may serve as an indicator for cardiac omega-3 fatty acid content**

**Metcalf et al. 2010, Am J Clin Nutr. 91(3); 528-34**
Increasing the Omega-3 Index reduces risk of both primary cardiac arrest and sudden cardiac death

Clinical Evidence – Cross Sectional
Primary Cardiac Arrest and the Omega-3 Index
Seattle PCA Study

90% reduction in risk

Odds Ratio

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Mean RBC EPA+DHA (%)</th>
</tr>
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<tbody>
<tr>
<td>Q1</td>
<td>3.3%</td>
</tr>
<tr>
<td>Q2</td>
<td>4.3%</td>
</tr>
<tr>
<td>Q3</td>
<td>5.0%</td>
</tr>
<tr>
<td>Q4</td>
<td>6.5%</td>
</tr>
</tbody>
</table>

*p < 0.05 vs Q1

Clinical Evidence – Prospective
Sudden Cardiac Death and the Omega-3 Index
Physicians' Health Study

90% reduction in risk

Relative Risk

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Mean Blood Omega-3 FA (%)</th>
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<tbody>
<tr>
<td>Q1</td>
<td>3.6%</td>
</tr>
<tr>
<td>Q2</td>
<td>4.8%</td>
</tr>
<tr>
<td>Q3</td>
<td>5.6%</td>
</tr>
<tr>
<td>Q4</td>
<td>6.9%</td>
</tr>
</tbody>
</table>

p for trend = 0.001

Adapted from Siscovick et al., JAMA 1995, 274; 1363-7

The Omega-3 Index and risk for total mortality - Women’s Health Initiative Memory Study with a median 14.9 years follow-up

31% lower risk for death with an Omega-3 Index of >8% vs <4% (p=0.012)

Harris et al., J Clin Lipidol. 2017, 11; 250-9
Global Omega-3 Index by country

Circulating EPA+DHA levels taken from

- 24,129 individual subjects
- 54 countries
- 398 data sets
- Converted to Omega-3 Index equivalents based on Stark et al.

Stark et al., Prog Lipid Res. 2016, 63; 132-52
Summary

• The purified krill oil phospholipid preparation had a marked beneficial effect on several risk factors for cardiovascular disease in dyslipidemic non-human primates by reducing total cholesterol, LDL-c and triglycerides, and increasing HDL-c
• A significant increase in Omega-3 Index was observed
• As a result of the increase of omega-3 fatty acids in erythrocyte membranes, omega-6 fatty acids were reduced. Saturated, mono-unsaturated and trans fatty acids were mostly unchanged
• Review of meta-analysis of human krill oil studies on blood lipid modification indicate the same trends as seen in the non-human primate study but the amount of data is still sparse
• The Omega-3 Index seems to have a remarkable effect on risk for sudden cardiac death
• Populations in most countries seem to have an Omega-3 Index below what is considered desireable
Acknowledgements

• Crown Bioscience (Taicang), Inc. for an excellent performance of the NHP study, OmegaQuant, SD USA for analyzing the fatty acids in erythrocyte membranes and LipoScience Inc., NC USA for analyzing lipid parameters by NMR

• All researchers working in the Omega-3 scientific area who have published their work and therefore contributed some of the data used in this presentation

• Colleagues at Aker Biomarine, in particular our Chief scientist Dr. Nils Hoem, for contributions to the science performed by the company and for fruitful and stimulating scientific discussions
THANK YOU!