Emerging science on omega-3: A lipidomic view of omega-3 in health and disease

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04 May 2017

Health benefits of omega 3 fatty acids

- Can lower plasma triacylglycerol
- Can reduce the risk of metabolic syndrome and type 2 diabetes?
- Have anti-inflammatory properties
- Can lower systemic inflammation
- Can reduce the risk of heart disease
- AHA Science Advisory
- Can reduce the risk of age related cognitive decline?
- Preclinical vs clinical trials

Omega-3 fatty acids and incident type 2 diabetes

The overall pooled findings do not support either major harms or benefits of fish/seafood or EPA + DHA on development of DM, and suggest that ALA may be associated with modestly lower risk.

Reasons for potential heterogeneity of effects, which could include true biologic heterogeneity, publication bias, or chance, deserve further investigation.

Omega-3 Polyunsaturated Fatty Acid (Fish Oil) Supplementation and the Prevention of Clinical Cardiovascular Disease

- May reduce CHD death in patients with a prior history of CHD
- May reduce CHD death in patients with a history of heart failure
- Evidence supports the reduction of recurrent atrial fibrillation
- No effect on recurrent non-fatal MI
- No benefit in CVD outcomes in patients with or at risk of diabetes mellitus
- No benefit in recurrent stroke in patients with prior stroke
- Effects thought to be through a reduction of ischemia induced sudden cardiac death

Omega-3 fatty acids and incident type 2 diabetes: a systematic review and meta-analysis.
Wu JH¹, Micha R, Imamura F, Pan A, Biggs ML, Ajaz O, Djousse L, Hu FB, Mozaffarian D.

Omega-3 Polyunsaturated Fatty Acid (Fish Oil) Supplementation and the Prevention of Clinical Cardiovascular Disease: A Science Advisory from the American Heart Association. Siscovick et al. American Heart Association Nutrition Committee of the Council on Lifestyle and Cardiometabolic Health; Council on Epidemiology and Prevention; Council on Cardiovascular Disease in the Young; Council on Cardiovascular and Stroke Nursing; and Council on Clinical Cardiology.
How do Omega-3 fatty acids exert health benefits

Omega-3 fatty acid metabolites
- Protectins, resolvins and maresins have demonstrated anti-inflammatory effects
  - Can modulate the immune response and lower systemic inflammation
Can influence other factors such as oxidation
- Can be oxidised and so protect other lipids/proteins (oxidative stress)
Direct effect on cell receptors
- PPAR and other transcription factors
Effects on cell behaviour mediated by composition of cell membranes
- Influence cell membrane fluidity (lipid rafts)
  - Via incorporation into phospholipid pools

Plasma lipidomics at Baker Institute

Clinical lipid measures
- Cholesterol, HDL-C, triglycerides, LDL-C
Lipid composition of plasma
- Cholesteryl esters
- Di- and Triacylglycerols
- Phospholipids
- Ceramides
- Glycosphingolipids
- Sphingomyelin
- Modified lipids (oxidized, glycated)
- Lysolipids
- Free fatty acids

10,000 different lipids in humans (most will be in plasma at some level (>1000 abundant species)

Metabolomics Laboratory (Analytical platforms)

Sciex API 4000 Q/TRAP triple quadrupole mass spectrometer
- Agilent 1200 HPLC system
- Column = Agilent Zobax C-18 eclipse (1.8 uM x 50 mM)

Agilent 6490 triple quadrupole mass spectrometer
- Agilent 1290 HPLC system
- Column = Agilent Zobax C-18 eclipse plus (1.8 uM x 50 mM)

Metabolomics Laboratory (High throughput lipidomics)

Lipid extraction (10µL plasma)\(^1\)
- Addition of stable isotope/non-physiological standards
- Single phase BuOH/MeOH
- Centrifuge (supernatant)
- LC-MS/MS

Lipid quantification (relative)\(^2\)
- LC ESI-MS/MS
- Stable isotope dilution (non-physiological)
- Multiple reaction monitoring (scheduled)
- 350 lipid species (10 min)

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**Plasma lipid MRM experiment (20min LC gradient)**

- Current capabilities >600 lipid species in 15 min

**Separation of isobaric and isomeric species**

**Phospholipids** Separation of acyl / alkyl / alkenyl species of the same nominal mass

**High throughput lipidomic profile**

<table>
<thead>
<tr>
<th>Lipid class/subclass</th>
<th>No. of species</th>
<th>Parent ion</th>
<th>Daughter ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sphingosylceramide (SphCer)</td>
<td>6</td>
<td>[M+H]^+</td>
<td>264.3 m/z</td>
</tr>
<tr>
<td>Ceramide (Cer)</td>
<td>41</td>
<td>[M+H]^+</td>
<td>Sphingosyl specific m/z</td>
</tr>
<tr>
<td>Monohexosylceramide (MHG)</td>
<td>6</td>
<td>[M+H]^+</td>
<td>264.3 m/z</td>
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<tr>
<td>Dihexosylceramide (DHC)</td>
<td>6</td>
<td>[M+H]^+</td>
<td>264.3 m/z</td>
</tr>
<tr>
<td>Phosphatidylethanolamine (PE)</td>
<td>68</td>
<td>[M+H]^+</td>
<td>184.1 m/z</td>
</tr>
<tr>
<td>Sphingosylphosphatidylethanolamine (SPE)</td>
<td>22</td>
<td>[M+H]^+</td>
<td>184.1 m/z</td>
</tr>
<tr>
<td>Alkenylphosphatidylethanolamine (PAPE)</td>
<td>24</td>
<td>[M+H]^+</td>
<td>184.1 m/z</td>
</tr>
<tr>
<td>Sphingosylphosphatidylinositol (SPI)</td>
<td>56</td>
<td>[M+H]^+</td>
<td>184.1 m/z</td>
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<tr>
<td>Sphingosylphosphatidylcholine (SPC)</td>
<td>10</td>
<td>[M+H]^+</td>
<td>104.1 m/z</td>
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<tr>
<td>Sphingosylphosphatidylethanolamine (SPPE)</td>
<td>4</td>
<td>[M+H]^+</td>
<td>104.1 m/z</td>
</tr>
<tr>
<td>Sphingosylphosphatidylcholine (SPC)</td>
<td>36</td>
<td>[M+H]^+</td>
<td>NL, 141.0 Da</td>
</tr>
<tr>
<td>Sphingosylphosphatidylethanolamine (SPPE)</td>
<td>14</td>
<td>[M+H]^+</td>
<td>NL, 141.0 Da</td>
</tr>
<tr>
<td>Sphingosylphosphatidylcholine (SPC)</td>
<td>14</td>
<td>[M+H]^+</td>
<td>NL, 141.0 Da</td>
</tr>
<tr>
<td>Cholesteryl ester derivatives</td>
<td>18</td>
<td>[M-NH4]^+</td>
<td>367.3 m/z</td>
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<tr>
<td>Acylcarnitine (AC)</td>
<td>14</td>
<td>[M+H]^+</td>
<td>85.1 m/z</td>
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<tr>
<td>Dicarboxylic acid (DC)</td>
<td>20</td>
<td>[M+H]^+</td>
<td>NL, fatty acid</td>
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<tr>
<td>Triacylglycerol (TAG)</td>
<td>44</td>
<td>[M+H]^+</td>
<td>NL, fatty acid</td>
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<tr>
<td>Alkyl-diaclylglycerol (ADG)</td>
<td>3</td>
<td>[M+H]^+</td>
<td>NL, fatty acid</td>
</tr>
<tr>
<td>TOTAL</td>
<td>602</td>
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</tr>
</tbody>
</table>
Obesity, Impaired Glucose Tolerance and Diabetes

Australia has an obesity epidemic

- Increase in type 2 diabetes
  - 7.6% in 2000 will rise to 11.4% by 2025
  - More than 1/3 individuals will develop diabetes
  - 1,000,000 new cases by 2025
  - Cost is in the $billions

Obesity is an important risk factor for type 2 diabetes
- not all obese individuals will go on to develop diabetes.

Changes in lipid metabolism play a key role in the development and progression of diabetes.

Lipidomics of Diabetes Prediabetes and Obesity

What is the lipid profile characteristic of:
- Diabetes?
- Prediabetes?
- Obesity?

What is the difference between:
- Diabetes and obesity?
- Diabetes and prediabetes?

Can we classify/predict diabetes from the lipid profile?

What is the influence of intervention (diet, drug) on the diabetogenic lipid profile?
Lipidomics of Diabetes Prediabetes and Obesity

**AusDiab cohort: Cross sectional study of newly diagnosed patients at baseline**

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Sex (%F)</th>
<th>BMI</th>
<th>Systolic BP</th>
<th>Total Chol</th>
<th>HDL Chol</th>
<th>TRIGs</th>
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</thead>
<tbody>
<tr>
<td>NGT (n = 170)</td>
<td>60</td>
<td>48</td>
<td>26.1</td>
<td>135</td>
<td>5.7</td>
<td>1.5</td>
<td>1.3</td>
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<tr>
<td>(±/13)</td>
<td>(±/3.1)</td>
<td>(±/21)</td>
<td>(±/1.0)</td>
<td>(±/0.4)</td>
<td>(±/0.6)</td>
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</tr>
<tr>
<td>IGT/IFG (n=64)</td>
<td>66</td>
<td>53</td>
<td>26.8</td>
<td>143</td>
<td>6.1</td>
<td>1.4</td>
<td>2.0</td>
</tr>
<tr>
<td>(±/11)</td>
<td>(±/3.2)</td>
<td>(±/23)</td>
<td>(±/1.1)</td>
<td>(±/0.5)</td>
<td>(±/1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic (n = 117)</td>
<td>62</td>
<td>51</td>
<td>28.2</td>
<td>144</td>
<td>6.0</td>
<td>1.3</td>
<td>2.2</td>
</tr>
<tr>
<td>(±/13)</td>
<td>(±/3.5)</td>
<td>(±/20)</td>
<td>(±/1.1)</td>
<td>(±/0.4)</td>
<td>(±/1.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Lipidomics of Diabetes Prediabetes and Obesity (Logistic regression analysis)**

**Lipid extraction**
- 10mL plasma
- Single phase CHCl₃ / MeOH / H₂O
- BuOH / MeOH / H₂O

**Multiple reaction monitoring (MRM)**
- 2 x LC ESI-MS/MS experiments
- 351 participants
- 337 lipid species in total
- 7 days
- 118,000 measurements

**282 known lipid species**
- 142 associated with Diabetes (vs. NGT, p<0.05)*
- 116 associated with Prediabetes (vs. NGT, p<0.05)*
- 58 associated with Obesity (vs.Non-obese, p<0.05)*
  - 108 associated with Obesity (vs. Non-obese, p<0.05)

* Corrected for multiple comparisons (Benjamini-Hochberg)

Lipids:
- dihydroceramide
- ceramide
- monohexosylceramide
- dhexosylceramide
- trihexosylceramide
- phosphadidylyceroline
- odd chain phosphatidylcholine
- alkylphosphatidylcholine
- alkenylphosphatidylcholine
- phosphatidylethanolamine
- phosphatidylserine
- phosphatidylinositol
- phosphatidylglycerol
- cholesterol ester
- diacylglycerol
- triacylglycerol
- serine + palmitate

Odds Ratio

ILSI SEA Region Seminar on Re-assessing Macronutrient Needs – Requirement, Quality and Health Impact, May 3-4, 2017, Bangkok, Thailand
Validation with San Antonio Family Heart Study (n=1200, 40 families)

Lipidomics of Diabetes Prediabetes and Obesity (Odds Ratio: Diabetes vs Obesity)

Lipidomics of Diabetes Prediabetes and Obesity (correlation to dietary n-3 PUFA)

Omega-3 and type 2 diabetes (Summary)

- Plasma lipid species are altered in obesity prediabetes and type 2 diabetes
  - Ceramides are positively associated with T2D
  - Alkenylphosphatidylcholines (plasmalogens) are negatively associated with T2D
- Omega-3 containing species correlate with dietary intake of omega-3 fatty acids
  - Distribution into different lipid pools likely to also be a contributing factor
- Omega-3 containing species are positively associated with type 2 diabetes
  - This may be a result rather than a cause of T2D

The plasma lipidomic profile provides a detailed view of lipid metabolism and can be used to assess the effect of dietary intervention studies on metabolic health.
Plasmalogens as a potential therapeutic for cardiometabolic disease

Plasmalogens and atherosclerosis

Polar head group

Anti-atherogenic
- anti-oxidant
- anti-inflammatory
- cholesterol efflux

Baker IDI clinical cohort (n=220)
- Plasmalogens negatively associated with stable and unstable coronary artery disease (Meikle et al, ATVB. 2011;31(11):2723-32)

San Antonio Family Heart Study (n=1200)
- Plasmalogens negatively associated with type 2 diabetes

LIPID / ADVANCE Study (n=10,000)
- Plasmalogens negatively associated with future CVE (CVD death)
  - particularly omega-3 species

Biosynthetic pathway of plasmalogens
Plasmalogen upregulation to prevent atherosclerosis in ApoE mice (Aliki Rasmiena)

Analysis

Lipid profiles of mouse plasma and heart
Sudan IV staining for lesions/plaque in aorta
Detection of inflammatory and oxidative markers in aorta and aortic sinus sections

Batyl alcohol supplementation increased plasmalogen levels in plasma

Batyl alcohol supplementation increased plasmalogen levels in heart

Data are median (interquartiles) of N = 9–10/group. *** indicates P<0.001
Assessment of aortic lesions

Batyl alcohol supplementation attenuated atherosclerosis in ApoE deficient mice

Data are mean ± SEM, expressed as % plaque area, n = 10/group. Data were analysed using student t-tests and compared to 0% BA treated group of the corresponding genotypes. *** indicates P < 0.001

Plasmalogen upregulation to prevent atherosclerosis in ApoE mice

Batyl alcohol containing diet successfully elevated plasmalogen concentration in plasma and heart

Plasmalogen up-regulation reduced atherosclerosis in ApoE−/− and ApoE−/−GPX−/−

Differential effects on inflammation and oxidative stress were observed in ApoE−/− and ApoE−/−GPX−/− mouse model

A lipidomic view of omega-3 in health and disease

Plasma lipidomics provide a composite measure of environmental (dietary) and genetic influences on metabolism

Plasma lipidomics can provide a detailed picture of metabolic health

Plasma lipidomics can predict risk of disease

Future studies will enable us to assess the effect of dietary interventions on plasma lipidomic profile and thereby on disease risk

Such studies may help guide dietary recommendations in the future
Acknowledgments

Baker Heart and Diabetes Institute
Metabolomics Lab

University of Indonesia
Kevin Aristyo Gunawan
Kevin Culham

Baker Heart and Diabetes Institute
Jonathan Shaw / Dianna Magliano
Bronwyn Kingwell / Melissa Formosa
Paul Nestel