Dietary modulation of oxidized linoleic acid metabolites

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This presentation does not represent any policy or position of the US Federal Government. It is solely the scientific opinion of the presenter.

Outline

Overview: Fatty acids and their autacoid derivatives

Do dietary n-6 fatty acids play a role in NASH?

Randomized trial: Targeted alteration of dietary n-3 and n-6 fatty acids for treatment of Chronic Daily Headache

Future Directions

Overview: biochemistry of n-3 and n-6 fatty acids
Essential Dietary Fats and their Bioactive Metabolites

Polyunsaturated fats in U.S. diets

US per capita consumption of vegetable oils in the 20th century
**Oxidized linoleic acid in NASH**
(Ariel Feldstein et al)

**Oxidized Fatty Acid Profiling by Mass Spectrometry**
- Liquid chromatography with on-line electrospray ionization tandem mass spectrometry (LC/ESI/MS/MS)
- Chiral mass spectrometry
- Quantification specific lipid oxidation products and their stereospecific isomers
**Human Protocol**

Clinical suspicion of NAFLD

Baseline → Liver tissue and blood were collected

- Liver histology (Blinded to OxFA levels)
  - Minimal changes (c)
  - Isolated steatosis (s)
  - NASH (n)

- Plasma OxFA levels using LC/ESI/MS/MS (Blinded to liver histology)

OxFA levels compared between three groups of study subjects (n = 73)

Feldstein et al. J. Lipid Res. July 14, 2010

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**Circulating OxFA Profile of Human NASH**

Feldstein et al. J. Lipid Res. July 14, 2010

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**Correlations Between OxNASH and Pathologist Diagnosis**

Feldstein et al. J. Lipid Res. July 14, 2010
Dietary linoleic acid in ASH
(Craig McClain et al)

The Type of Dietary Fat Modulates Intestinal Tight Junction Integrity, Gut Permeability, and Hepatic Toll-Like Receptor Expression in a Mouse Model of Alcoholic Liver Disease

Dietary modulation of oxidized linoleic acid metabolites
(Ramsden, Feldstein, Mann et al)
Targeted alteration of dietary n-3 and n-6 fatty acids for the treatment of chronic headaches: A randomized trial

Rationale for targeted dietary intervention

General model

Mechanisms linking n-3 & n-6 fatty acids to physical pain

Development of 2 putative anti-nociceptive dietary interventions

- H3-L6 intervention
  - Increase n-3 EPA and DHA
  - Reduce n-6 LA

- L6 intervention
  - Maintain low n-3 EPA and DHA intakes (typical of US)
  - Reduce n-6 LA and n-6 AA

MacIntosh BA, Ramsden CE et al. JIN 2012
**LA, EPA and DHA Consumption in Chronic Daily Headache Trial**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>n-6 LA*</th>
<th>n-3 EPA + DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>6.74</td>
<td>46 mg</td>
</tr>
<tr>
<td>H3-L6</td>
<td>2.51</td>
<td>1.482 mg</td>
</tr>
<tr>
<td>L6</td>
<td>2.40</td>
<td>76 mg</td>
</tr>
</tbody>
</table>

*LA intake is expressed as a percentage of daily food energy (%E). Median intakes assessed via six 24-hour dietary recalls administered on non-consecutive days.

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**Patient population**

**‘Chronic Daily Headache’**

Headache characteristics

- ≥ 15 headache days per month
- ≥ 4 headache hours per day

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**Trial Profile**

**Overview of Trial Design**

1. **Assessment for eligibility**
   - Total screened: n=91

2. **Randomisation**
   - Randomised: n=60

3. **Detailed trial intervention**
   - n-6 LA intervention: n=30
   - n-3 EPA + DHA intervention: n=30

4. **Follow-up**
   - Outcome evaluation: n=60
   - Completed: n=56
   - Withdrawn: n=4
   - Lost to follow-up: n=0

5. **Intention to treat analysis**
   - n=60

6. **Final report**
   - Macintosh BA, Ramsden CE, Mann JD et al., BJN 2012

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**Chronic Daily Headache**

Characteristics

- Chronic daily headache: ≥ 15 headache days per month and ≥ 4 headache hours per day
Diets altered erythrocyte fatty acids in a manner predicted to reduce physical pain

<table>
<thead>
<tr>
<th>Lipid Mediator</th>
<th>H3-L6 Intervention</th>
<th>L6 Intervention</th>
<th>P-value (Between-group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA plus DHA (≥3 longs)</td>
<td>+9.7</td>
<td>+10.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ω-3 Linoleic acid</td>
<td>-12.1</td>
<td>-13.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ω-6 Arachidonic acid</td>
<td>-14.0</td>
<td>-7.7</td>
<td>0.01</td>
</tr>
<tr>
<td>ω-6 in n6:3 ratio</td>
<td>-21.0</td>
<td>-4.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Ramsden CE, Mann JD et al., Trials 2011, Pain 2013

H3-L6 intervention produced greater pain reduction

Diet-induced changes in anti- and pronociceptive lipid mediators

Between-group comparisons:
- p<.001
- p<.02
- p<.01
- p<.02
- p<.01

0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100

% change (12 weeks)

HIT-6
Severe Headache days
Headache days/month
Headache hours/day
Diet-induced changes in anti- and pronociceptive lipid mediators

<table>
<thead>
<tr>
<th></th>
<th>H3-L6</th>
<th>L6</th>
<th>Between-arms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-nociceptive mediators</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA oxidation product 16-HEPE</td>
<td>+0.12</td>
<td>+0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DHA oxidation products</td>
<td></td>
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<tr>
<td>Resolvin pathway precursors</td>
<td></td>
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<tr>
<td>Endocannabinoids</td>
<td></td>
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<tr>
<td>Putative Endovanilloids</td>
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<tr>
<td>Pronociceptive mediators</td>
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<tr>
<td>LA oxidation products</td>
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<td></td>
<td></td>
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<tr>
<td>Resolvin</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Prostaglandins</td>
<td></td>
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<tr>
<td>Thromboxanes</td>
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Headache Results Summary

The H3-L6 intervention:
Produced statistically significant, clinically relevant improvements in:
- Headache hours per day
- Severe headache days
- Quality of life

Produced marked alterations in circulating n-3 and n-6 derived:
- Eicosanoids
- Resolvin pathway precursors
- Endocannabinoids
- Putative Endovanilloids

Limitations

These findings should be replicated in a larger trial.

Targeted fatty acids were not altered as independent variables.

The clinical effects of the H3-L6 intervention should also be evaluated in comparison to a control intervention providing habitual intakes of the targeted dietary fatty acids.

Could not establish whether comparable diet-induced biochemical alterations are possible in other tissues implicated headache pathogenesis.
Future directions (NASH)

Determine whether dietary LA lowering:
1. Reduces OXLAMs in liver
2. Protects from development/progression NASH

Delineate molecular mechanisms linking dietary LA and OXLAMs to NASH

END

Diet-induced changes in N-acyl ethanolamine and glycerol ester endocannabinoids

Ramsden, Makriyannis, Hibbeln, et al. unpublished
### Model depicting diet-induced alterations in endocannabinoids

![Model depicting diet-induced alterations in endocannabinoids](image)

### Diet-induced changes in plasma endocannabinoids

<table>
<thead>
<tr>
<th></th>
<th>n-3 family</th>
<th></th>
<th></th>
<th></th>
<th>n-6 family</th>
<th></th>
<th></th>
<th></th>
<th>Other</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endocannabinoids (ng/mL)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>2-DHG</td>
<td>181 (119, 242)</td>
<td>264 (187, 400)</td>
<td>+65</td>
<td>&lt;0.001</td>
<td>Baseline</td>
<td>143 (120, 178)</td>
<td>172 (126, 226)</td>
<td>+17</td>
<td>0.14</td>
<td>0.001</td>
</tr>
<tr>
<td>Baseline</td>
<td>DHA-EA</td>
<td>0.43 (0.29, 0.62)</td>
<td>0.80 (0.55, 1.20)</td>
<td>+99</td>
<td>&lt;0.001</td>
<td>Baseline</td>
<td>0.43 (0.29, 0.56)</td>
<td>0.45 (0.37, 0.56)</td>
<td>+14</td>
<td>0.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline</td>
<td>2-AG</td>
<td>908 (628, 1068)</td>
<td>557 (444, 760)</td>
<td>-25</td>
<td>0.001</td>
<td>Baseline</td>
<td>637 (492, 856)</td>
<td>703 (478, 963)</td>
<td>+3</td>
<td>0.50</td>
<td>0.004</td>
</tr>
<tr>
<td>Baseline</td>
<td>AEA</td>
<td>0.49 (0.38, 0.53)</td>
<td>0.44 (0.34, 0.51)</td>
<td>-3</td>
<td>0.51</td>
<td>Baseline</td>
<td>0.47 (0.35, 0.58)</td>
<td>0.50 (0.43, 0.61)</td>
<td>+1</td>
<td>0.36</td>
<td>0.059</td>
</tr>
<tr>
<td>Baseline</td>
<td>OEA</td>
<td>2.74 (2.07, 3.30)</td>
<td>2.71 (2.05, 3.42)</td>
<td>+9</td>
<td>0.48</td>
<td>Baseline</td>
<td>2.82 (2.19, 3.45)</td>
<td>2.85 (2.23, 3.62)</td>
<td>+13</td>
<td>0.05</td>
<td>0.498</td>
</tr>
<tr>
<td>Baseline</td>
<td>DGE</td>
<td>3.05 (2.73, 3.45)</td>
<td>3.22 (2.67, 3.61)</td>
<td>+15</td>
<td>0.68</td>
<td>Baseline</td>
<td>3.30 (2.85, 3.81)</td>
<td>3.56 (2.84, 4.36)</td>
<td>+20</td>
<td>0.18</td>
<td>0.217</td>
</tr>
<tr>
<td>Baseline</td>
<td>DHA</td>
<td>3.27 (2.82, 3.67)</td>
<td>2.85 (2.40, 3.31)</td>
<td>-16</td>
<td>0.28</td>
<td>Baseline</td>
<td>2.85 (2.47, 3.28)</td>
<td>2.86 (2.52, 3.19)</td>
<td>+0</td>
<td>0.05</td>
<td>0.265</td>
</tr>
</tbody>
</table>

### Acknowledgements

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