Influence of dietary fatty acids on the endocannabinoid system

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endocannabinoids are bioactive lipids, derived from membrane arachidonic acid

Anandamide (AEA)

Arachidonoyl glycerol (2-AG)

CB₁

Immune system
T, B cells, monocytes spleen

CB₂
Central and peripheral targets of the endocannabinoid system and effects of overactivity

Brain

- Hypothalamus: hunger
- Nucleus accumbens: motivation to eat

Increased food intake
Increased fat storage

Peripheral tissues

- Adipose tissue
- Liver
- GI tract
- Muscle

- Insulin resistance
- HDL-cholesterol
- Triglycerides
- Glucose uptake
- Adiponectin

HDL: high-density lipoprotein

References:
Phospholipids

Sn-1

6%

20:4 arachidonic acid

anandamide

Sn-2

50%

20:4 arachidonic acid

2-AG

endocannabinoids
Influenced by the diet

Phospholipids

- Sn-1
  - 6%
  - 20:4 arachidonic acid
  - anandamide

- Sn-2
  - 50%
  - 20:4 arachidonic acid
  - 2-AG
  - endocannabinoids

Anandamide and 2-AG are endocannabinoids.
Dietary fatty acids modify endocannabinoid biosynthesis


Omega-3 fatty acids – health benefits

- Cardiovascular
  - HTG
  - Post-MI
  - Mixed Dyslipidemia
  - AF
  - CHF
  - Atherosclerosis
  - Hypertension

- CNS
  - Alzheimer's
  - Depression
  - Schizophrenia
  - Dementia
  - ADHD
  - Stroke

- Omega-3
  - Metabolic disorders
    - Diabetes
    - Obesity
    - Fatty Liver
  - Anti-inflammatory
    - Arthritis
    - Lupus
    - IBD
    - Asthma
    - Cystic fibrosis
    - Psoriasis
Does dietary omega-3, by modulating the endocannabinoid system, affect metabolic syndrome?

- Obesity model – Zucker rats
- Clinical studies in obese and overweight subjects
Zucker rats: a model of visceral obesity

Zucker fa/fa rats exhibit an inactivating mutation in the leptin receptor, inducing hyperphagia and resulting, from the fourth week of age, in visceral obesity and ectopic lipid accumulation.
Adipose tissue

Subcutaneous Adipose tissue
- Sensitive to PPARgamma
- Sensitive to insulin

TAG accumulation
Pre-adipocyte differentiation

Post-prandial TAG accumulation
NEFA release In fasted state

Visceral adipose tissue
- Sensitive to PPARgamma
- Sensitive to insulin

TAG accumulation
In post-prandial
Steady NEFA release

+ TAG accumulation
In tissue (muscle)

+ NEFA accumulation
In liver via portal vein

+ tissue fatty acid uptake

+beta-ox accumulation

Estrogens
≈ 80%

Androgens
≈ 20%

Glucocorticoids

Adrenergic System

Sensitive to PPARgamma

Sensitive to insulin
Endocannabinoids

Subcutaneous Adipose tissue
- EC
- Low PPARgamma activation
- Minor TAG deposition

Visceral adipose tissue
- EC
- High PPAR gamma activation
- Increased TAG deposition
- NEFA release

Ectopic fat accumulation
Obese Zucker Rats: 250g, 4 week-treatment with omega-3 (EPA+DHA 0.5% in the diet) in two forms:

- **Fish Oil**, Omega-3 in TAG form
- **Krill Oil**, a high content of PC-bound n-3 LCPUFAs (30-60%)
### Dietary fatty acid composition (g/100g diet)

The diets were based on the AIN-93G formulation, with substitution of soybean oil with a blend of oils (rapeseed oil, sunflower oil, coconut oil, and linseed oil).

4 week-treatment with omega-3 (EPA+DHA 0.5% in the diet equivalent to 0.8% of energy/day)

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>FO</th>
<th>KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:3(n-3)</td>
<td>0.26</td>
<td>0.26</td>
<td>0.29</td>
</tr>
<tr>
<td>18:4(n-3)</td>
<td>0.00</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>0.00</td>
<td>0.29</td>
<td>0.30</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>0.00</td>
<td>0.18</td>
<td>0.14</td>
</tr>
<tr>
<td>Total(n-3)</td>
<td>0.26</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>2.07</td>
<td>2.23</td>
<td>2.22</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Total(n-6)</td>
<td>2.07</td>
<td>2.24</td>
<td>2.23</td>
</tr>
<tr>
<td>(n-6):(n-3)</td>
<td>7.85</td>
<td>2.86</td>
<td>2.76</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>2.34</td>
<td>2.72</td>
<td>2.25</td>
</tr>
<tr>
<td>TotalUFA</td>
<td>4.68</td>
<td>5.74</td>
<td>5.29</td>
</tr>
<tr>
<td>12:0</td>
<td>1.09</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>14:0</td>
<td>0.39</td>
<td>0.20</td>
<td>0.38</td>
</tr>
<tr>
<td>16:0</td>
<td>0.58</td>
<td>0.78</td>
<td>1.09</td>
</tr>
<tr>
<td>18:0</td>
<td>0.22</td>
<td>0.21</td>
<td>0.19</td>
</tr>
<tr>
<td>20:0</td>
<td>0.03</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>TotalSFA</td>
<td>2.32</td>
<td>1.26</td>
<td>1.71</td>
</tr>
</tbody>
</table>

C = control
FO = fish oil
KO = krill oil

Batetta et al, J Nutr 2009
Visceral Adipose Tissue

Batetta et al, J Nutr 2009
Endocannabinoids activate PPARgamma

Subcutaneous adipose tissue

EC

high PPAR gamma activation

Minor TAG deposition

Visceral adipose tissue

EC

Low PPAR gamma activation
decreased TAG deposition

Lower NEFA release

Ectopic fat accumulation

Physiologic balance
FO=fish oil KO=krill oil

Liver Control diet

Liver Omega-3 diets

Batetta et al, J Nutr 2009
Clinical study in overweight and obese subjects

- **Study:** A Randomized, Controlled, Double-Blind Study to Evaluate the Safety and Tolerability of Krill Oil in Men and Women

- **Subjects:** Men and women 35-64 years of age (inclusive) with waist circumference $\geq 102$ cm (men) or $\geq 88$ cm (women).

- **Number of subjects:** 25 per group

- **Treatment:** 2g krill oil, 2g fish oil or 2g olive oil (control)

- **Duration:** 4 weeks

*Maki et al., Nutr Res 2009*
Dietary krill oil lowers (4 weeks) plasma 2-AG levels in human obese subjects (BMI~30, mostly women, N=19-23)

*Banni et al. Nutr Metab 2011*
Human Study

2g/d dietary krill oil (4 weeks) decreased plasma 2-AG levels in human obese subjects (BMI~30, mostly women, N=19-23)

Banni et al. Nutr Metab 2011
Single-centre, open label, pilot study on the effect of Krill powder on triglyceridemia and endocannabinoids in hypertriglyceridemic, mildly obese subjects (n=11)

Treatment:
4g Krill Powder
For 24 weeks

<table>
<thead>
<tr>
<th></th>
<th>% in powder</th>
<th>Content in 4 g krill powder (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>34</td>
<td>1360</td>
</tr>
<tr>
<td>Total lipids</td>
<td>61.8</td>
<td>2472</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>31.5</td>
<td>1260</td>
</tr>
<tr>
<td>Total PLs</td>
<td>25.4</td>
<td>1016</td>
</tr>
<tr>
<td>Total omega-3</td>
<td>13.7</td>
<td>548</td>
</tr>
<tr>
<td>Total omega-6</td>
<td>1.4</td>
<td>56</td>
</tr>
<tr>
<td>Saturated FAs</td>
<td>17.9</td>
<td>716</td>
</tr>
<tr>
<td>Total PUFAs</td>
<td>15.6</td>
<td>624</td>
</tr>
<tr>
<td>Total MUFAs</td>
<td>13.2</td>
<td>528</td>
</tr>
<tr>
<td>EPA</td>
<td>6.7</td>
<td>268</td>
</tr>
<tr>
<td>DHA</td>
<td>3.3</td>
<td>132</td>
</tr>
</tbody>
</table>

Berge et al. Lipids Health Dis In Press
Sheep cheese naturally enriched in α-linolenic, conjugated linoleic and vaccenic acids improves the lipid profile and reduces anandamide in the plasma of hypercholesterolaemic subjects

Stefano Pintus¹, Elisabetta Murru², Gianfranca Carta², Lina Cordeddu², Barbara Batetta², Simonetta Accossu², Danila Pistis¹, Sabrina Uda², Maria Elena Ghiani², Marcello Mele³, Pierlorenzo Secchiari³, Guido Almerighi⁴, Paolo Pintus¹ and Sebastiano Banni²*

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³Dipartimento di Agronomia e Gestione dell’Agroecosistema, Università di Pisa, Pisa, Italy
⁴O. Obesità ASL 8, Cagliari, Italy

(Submitted 11 November 2011 – Final revision received 14 June 2012 – Accepted 19 June 2012 – First published online 24 August 2012)
## Cheese fatty acid composition

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>% in cheese fat</th>
<th>g/90 of cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTRL</td>
<td>ENCH</td>
</tr>
<tr>
<td>total SFA</td>
<td>59.3</td>
<td>45.9</td>
</tr>
<tr>
<td>short chain (c4-c10)</td>
<td>16.6</td>
<td>11.3</td>
</tr>
<tr>
<td>c12:0</td>
<td>2.9</td>
<td>1.8</td>
</tr>
<tr>
<td>c14:0</td>
<td>8.5</td>
<td>6.1</td>
</tr>
<tr>
<td>c16:0</td>
<td>20.5</td>
<td>16.0</td>
</tr>
<tr>
<td>c18:0</td>
<td>10.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Total cis MUFA</td>
<td>19.0</td>
<td>21.2</td>
</tr>
<tr>
<td>c16:1n-9</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>c18:1n-9</td>
<td>18.6</td>
<td>20.9</td>
</tr>
<tr>
<td>Total trans MUFA</td>
<td>3.4</td>
<td>10.6</td>
</tr>
<tr>
<td>c18:1 t11 (VA)</td>
<td>1.7</td>
<td>6.3</td>
</tr>
<tr>
<td>Total PUFA n-6</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>c18:2n-6 (LA)</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Total PUFA n-3</td>
<td>0.6</td>
<td>2.1</td>
</tr>
<tr>
<td>c18:3n-3 (ALA)</td>
<td>0.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Total trans PUFA</td>
<td>0.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Total CLA</td>
<td>1.0</td>
<td>2.8</td>
</tr>
<tr>
<td>c9,t11 CLA</td>
<td>0.8</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Mele et al. Int Dairy J. 2011
Percentage of fatty acid classes in enriched cheese replacing saturated fatty acids

- tot CLA: 13%
- MUFA: 16%
- tot PUFA n-3: 11%
- trans UFA: 9%
- trans MUFA: 51%

Pintus et al. Br J Nutr 2013
This study was a 3-week, randomized, double blind, controlled, cross over clinical trial, conducted at the State Hospital Brotzu in Cagliari, Italy.

Reduplicated with 45g of enriched or control cheese.

Pintus et al. Br J Nutr 2013
Vaccenic Acid

Alpha Linolenic Acid
\((c_9,c_{12},c_{15}-C_{18:3})\)

Linoleic Acid
\((c_9,c_{12}-C_{18:2})\)

CLA

Rumine

\(\Delta^9\) desaturase

\(c_9,\Delta^9-C_{18:2}\)

\((\Delta^9)C_{18:1}\)

\((c_9,\Delta^9-C_{18:2})\)

Pintus et al. Br J Nutr 2013
Alpha Linolenic acid

\[\omega-3\]

18:3 alpha linolenic

\[\Delta6\text{ Desaturase}\]

18:4

Elongase

20:4

\[\Delta5\text{ Desaturase}\]

20:5

EPA

24:5

\[\Delta6\text{ Desaturase}\]

24:6

\[\beta\text{-ox. In peroxisome}\]

22:6

DHA

Pintus et al. Br J Nutr 2013
Omega-6/omega-3

\[ \begin{align*}
\omega-3 & \quad 18:3 \\
& \quad \downarrow \\
& \quad 18:4 \\
& \quad \downarrow \\
& \quad 20:4 \\
& \quad \downarrow \\
& \quad 20:5 \\
& \quad \downarrow \\
& \quad 24:5 \\
& \quad \downarrow \\
& \quad 24:6 \\
& \quad \downarrow \\
& \quad 22:6 \\
\end{align*} \]

\[ \begin{align*}
\omega-6 & \quad 18:2 \\
& \quad \downarrow \\
& \quad 18:3 \\
& \quad \downarrow \\
& \quad 20:3 \\
& \quad \downarrow \\
& \quad 20:4 \\
& \quad \downarrow \\
& \quad 24:4 \\
& \quad \downarrow \\
& \quad 24:5 \\
& \quad \downarrow \\
& \quad 22:5 \\
\end{align*} \]

\[ \begin{align*}
\Delta 6 \text{ Desaturase} & \\
\Delta 5 \text{ Desaturase} & \\
\text{Elongase} & \\
\text{EPA} & \\
\Delta 6 \text{ Desaturase} & \\
\text{B-ox. In peroxisome} & \\
\text{DHA} & \\
\end{align*} \]

Pintus et al. Br J Nutr 2013
Phospholipids

Sn-1

20:4

Sn-2

20:4

anandamide

2-AG

endocannabinoids

Pintus et al. Br J Nutr 2013
<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>REGCH</th>
<th>ENCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (Kg/m²)</td>
<td>26.64 ± 2.83</td>
<td>26.30 ± 3.18</td>
<td>26.03 ± 3.21</td>
</tr>
<tr>
<td>Tot chol. (mg/dL)</td>
<td>243.52 ± 18.54</td>
<td>256.29 ± 31.96</td>
<td>230.80 ± 18.41</td>
</tr>
<tr>
<td>C-LDL (mg/dL)</td>
<td>165.88 ± 16.2a</td>
<td>170.55 ± 26.03a</td>
<td>154.79 ± 13.26b</td>
</tr>
<tr>
<td>C-HDL (mg/dL)</td>
<td>55.36 ± 9.46a</td>
<td>61.84 ± 11.48b</td>
<td>56.54 ± 10.42a</td>
</tr>
<tr>
<td>C- non HDL (mg/dL)</td>
<td>188.17 ± 19.44a</td>
<td>188.74 ± 41.95a</td>
<td>174.27 ± 18.38b</td>
</tr>
<tr>
<td>tot/HDL</td>
<td>4.54 ± 0.98</td>
<td>4.25 ± 0.81</td>
<td>4.23 ± 0.92</td>
</tr>
<tr>
<td>TAG (mg/dL)</td>
<td>111.81 ± 52.06</td>
<td>120.29 ± 87.92</td>
<td>111.71 ± 69.70</td>
</tr>
<tr>
<td>Glycemia (mg/dL)</td>
<td>99.33 ± 12.17</td>
<td>96.97 ± 8.05</td>
<td>98.98 ± 12.85</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.99 ± 0.16</td>
<td>0.96 ± 0.13</td>
<td>0.97 ± 0.15</td>
</tr>
<tr>
<td>Uricemia (mg/dL)</td>
<td>5.14 ± 1.13</td>
<td>5.14 ± 3.02</td>
<td>4.89 ± 1.06</td>
</tr>
</tbody>
</table>
Conclusions

- Our data show that a decrease of substrate for endocannabinoid biosynthesis by n-3 LCPUFAs, reduces endocannabinoids and their activities in promoting metabolic syndrome.

- However, not only n-3 LCPUFAs, but any dietary strategy to increase plasma n-3/n-6 LCPUFAs may reduce an overactive endocannabinoid system, as it occurs in obese subjects.

- Dietary fatty acids, not as a single but in a concerted action, may play a significant role in the physiological control of the endocannabinoid system and thereby controlling lipid and energy metabolism.
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