Origin of Conjugated Linoleic Acids

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“Functional Foods” and Health Promotion

“any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains”

Institute of Medicine, NAS 1994
Partial List of Health Effects of CLA

**Biological Effect**

Anticarcinogenic effects (*in vivo* and *in vitro*)
Antiatherogenic properties
Altered nutrient partitioning and lipid metabolism
Antidiabetic (type II) and reduced hyperglycemia
Immune modulation
Improved bone mineralization

Bauman et al., 2001
CLA Isomers in Ruminant Fat

- *cis/trans, trans/trans, and cis/cis* isomers for:
  - 7,9, 9,11, 11,13
  - 8,10, 10,12, 12,14
- Many isomers identified in milk fat (n = 24) and beef fat (n = 14)
- *cis*-9, *trans*-11 in greatest abundance
- *trans*-7, *cis*-9 second in abundance
- *trans*-10, *cis*-12 increases under certain dietary situations

CLA Isomers in Supplement

- Four isomers in typical animal supplement (*trans*-8, *cis*-10; *cis*-9, *trans*-11; *trans*-10, *cis*-12; *cis*-11, *trans*-13)
- Two isomers in some human supplements (*cis*-9, *trans*-11; *trans*-10, *cis*-12)
### Milk Fat CLA Isomers

<table>
<thead>
<tr>
<th>Isomers</th>
<th>(% total CLA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>cis/trans isomers</strong></td>
<td></td>
</tr>
<tr>
<td>9,11</td>
<td>76.5</td>
</tr>
<tr>
<td>7,9</td>
<td>6.7</td>
</tr>
<tr>
<td>10,12</td>
<td>1.1</td>
</tr>
<tr>
<td>12,14</td>
<td>0.8</td>
</tr>
<tr>
<td>11,13</td>
<td>0.4</td>
</tr>
<tr>
<td>8,10</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>cis, cis isomers</strong></td>
<td>4.8</td>
</tr>
<tr>
<td><strong>trans, trans isomers</strong></td>
<td>9.4</td>
</tr>
</tbody>
</table>

Bauman et al. 2000
Lipid Metabolism in the Rumen

- microbial hydrolysis of dietary triglycerides
- microbial biohydrogenation of free fatty acids
- passage from rumen as free fatty acids (~85%) or as microbial phospholipids (~15%)
Pathways for Rumen Biohydrogenation

Linolenic Acid
\( \text{cis-9, cis-12, cis-15 } \text{C}_{18:3} \)

\( \text{(Group A & B)} \)

\( \text{cis-9, trans-11, cis-15 } \text{C}_{18:3} \)

\( \text{(Group A & B)} \)

\( \text{trans-11, cis-15 } \text{C}_{18:2} \)

\( \text{(Group B)} \)

\( \text{trans-15 or cis-15 } \text{C}_{18:1} \)

\( \text{(Group B)} \)

\( \text{Stearic Acid } \text{C}_{18:0} \)

Linoleic Acid
\( \text{cis-9, cis-12 } \text{C}_{18:2} \)

\( \text{(Group A & B)} \)

\( \text{cis-9, trans-11 } \text{CLA} \)

\( \text{(Group A & B)} \)

\( \text{trans-11 } \text{C}_{18:1} \)

\( \text{(Group A)} \)

\( \text{trans-11 } \text{C}_{18:1} \)

\( \text{(Group B)} \)

Griinari and Bauman, 1999
## Dietary Factors Which Affect CLA in Milk Fat

<table>
<thead>
<tr>
<th>Dietary Factor</th>
<th>Effect on CLA Content of Milk Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipid Substrate</strong></td>
<td></td>
</tr>
<tr>
<td>Unsaturated vs saturated fat</td>
<td>Increased by addition of unsaturated fat</td>
</tr>
<tr>
<td>Type of plant oil</td>
<td>Greatest with oils high in C18:2</td>
</tr>
<tr>
<td>Level of plant oil</td>
<td>Dose dependent increase</td>
</tr>
<tr>
<td>Ca salts of plant oils</td>
<td>Increased as with free oils</td>
</tr>
<tr>
<td>Fat in animal byproducts</td>
<td>Minimal effect</td>
</tr>
<tr>
<td>High oil plant feeds</td>
<td></td>
</tr>
<tr>
<td>high oil corn</td>
<td>Minimal effect</td>
</tr>
<tr>
<td>soybeans</td>
<td>Heat processing will increase</td>
</tr>
<tr>
<td>rapeseed vs soybean</td>
<td>Similar effect</td>
</tr>
<tr>
<td><strong>Modifiers of Biohydrogenation</strong></td>
<td></td>
</tr>
<tr>
<td>Forage:concentrate ratio</td>
<td>Increased with high ratio</td>
</tr>
<tr>
<td>Non structural carbohydrate level</td>
<td>Minor effect (possible oil x NSC interaction)</td>
</tr>
<tr>
<td>Restricted feeding</td>
<td>Increased with restricted</td>
</tr>
<tr>
<td>Fish oils</td>
<td>Greater increase than with plant oils</td>
</tr>
<tr>
<td>Monensin -ionophore</td>
<td>Variable effect</td>
</tr>
<tr>
<td>Dietary buffers</td>
<td>Little effect</td>
</tr>
<tr>
<td><strong>Combination</strong></td>
<td></td>
</tr>
<tr>
<td>Pasture vs conserved forages</td>
<td>Higher on pasture</td>
</tr>
<tr>
<td>Growth stage of forage</td>
<td>Increased with less mature forage</td>
</tr>
</tbody>
</table>

Bauman et al. 2001
Effects of Feeding Plant Oils on Milk Fat CLA

Chouinard et al. 2001
Pathways for Rumen Biohydrogenation

Linolenic Acid
\[ cis-9, \ cis-12, \ cis-15 \text{ C}_{18:3} \]
- (Group A & B)
  - \[ cis-9, \ trans-11, \ cis-15 \text{ C}_{18:3} \]
    - (Group A & B)
      - \[ trans-11, \ cis-15 \text{ C}_{18:2} \]
        - (Group B)
          - \[ trans-15 \text{ or cis-15 C}_{18:1} \]
            - (Group B)
              - Stearic Acid \[ C_{18:0} \]

Linoleic Acid
\[ cis-9, \ cis-12 \text{ C}_{18:2} \]
- (Group A & B)
  - \[ cis-9, \ trans-11 \text{ CLA} \]
    - (Group A & B)
      - \[ trans-11 \text{ C}_{18:1} \]
        - (Group B)
          - Stearic Acid \[ C_{18:0} \]

Grinari and Bauman, 1999
Relationship Between \textit{cis}-9, \textit{trans}-11 CLA and \textit{trans}-11 C_{18:1} in milk fat

\[ y = 0.30x + 0.22 \]

\[ R^2 = 0.61 \]

Peterson et al., 2002
\[ \Delta^9-\text{Desaturase} \]

\[
\begin{align*}
\text{NAD(P)H} & \xrightarrow{\text{Cyt } b_5 \text{ Reductase (FAD)}} \text{NAD(P)}^+ \\
\text{NAD(P)}^+ & \xrightarrow{\text{Cyt } b_5 \text{ Reductase (FADH}_2\text{)}} 2 \text{ Cyt } b_5 \text{ Fe}^{2+} \\
2 \text{ Cyt } b_5 \text{ Fe}^{2+} & \xrightarrow{\Delta^9-\text{Desaturase}} \text{cis-9, trans-11 } \text{C}_{18:2}\text{-CoA} + \text{H}_2\text{O} \\
2 \text{ Cyt } b_5 \text{ Fe}^{3+} & \xrightarrow{\Delta^9-\text{Desaturase}} \text{trans-11 } \text{C}_{18:1}\text{-CoA} + \text{O}_2
\end{align*}
\]

Sterculic Acid (cyclopropene fatty acid)

\[
\begin{align*}
\text{C} & \xrightarrow{9} \text{C} \xrightarrow{10} \text{C} \\
\text{-C-C=C-C-}
\end{align*}
\]
Sterculia foetida

Sterculia nut
41% oil
Sterculic oil
62% cyclopropenoic acids
trans C_{18:1} and CLA during Sterculic Oil Infusion

Corl et al., 1998
Desaturase Pairs *(cis-9 double bond)*

*trans*-11 \( \text{C}_{18:1} \) / *cis*-9, *trans*-11 CLA

\( \text{C}_{18:0} \) / \( \text{C}_{18:1} \) (stearic / oleic)

\( \text{C}_{16:0} \) / \( \text{C}_{16:1} \) (palmitic / palmitoleic)

\( \text{C}_{14:0} \) / \( \text{C}_{14:1} \) (myristic / myristoleic)
## Importance of Endogenous Synthesis

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control Milk fat CLA (mg/g)</th>
<th>Endogenous Synthesis of c9, t11 CLA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Mixed Ration</td>
<td>4.2</td>
<td>64%</td>
<td>Griinari et al., 2000</td>
</tr>
<tr>
<td>Total Mixed Ration + PHVO&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5</td>
<td>&gt;</td>
<td>Corl et al., 2001</td>
</tr>
<tr>
<td></td>
<td>7.6</td>
<td>78%</td>
<td></td>
</tr>
<tr>
<td>Pasture</td>
<td>15.5</td>
<td>&gt;90%</td>
<td>Kay et al., 2002</td>
</tr>
</tbody>
</table>

<sup>a</sup>Estimated by use of sterculic oil as a source of cyclopropene fatty acids to block \( \Delta^9 \)-desaturase.

<sup>b</sup>Partially hydrogenated vegetable oil.
Rumen Tissues

cis-9, trans-11 CLA

cis-9, cis-12 C₁₈:₂

cis-9, trans-11 CLA

trans-11 C₁₈:₁ (Vaccenic Acid)

Stearic Acid C₁₈:₀

Tissues

cis-9, trans-11 CLA

trans-11 C₁₈:₁

Δ⁹-desaturase

Endogenous Synthesis of CLA

Bauman et al., 2000
Source of *trans*-7, *cis*-9 CLA

Corl et al., 2002
Source of CLA Isomers in Milk Fat

**cis-9, trans-11**
- endogenous synthesis (major)
- rumen origin (minor)

**trans-7, cis-9**
- endogenous synthesis

**other cis/trans, trans/trans, and cis/cis isomers**
- rumen origin
Rumen Biohydrogenation

linoleic acid
\((\text{cis-9, cis-12 C}_{18:2})\)

conjugated linoleic acid
\((\text{cis-9, trans-11 CLA})\)

\textit{trans-11 C}_{18:1}

stearic acid \((\text{C}_{18:0})\)

conjugated linoleic acid
\(\text{trans-10, cis-12 CLA}\)

\textit{trans-10 C}_{18:1}

stearic acid \((\text{C}_{18:0})\)

Griinari and Bauman, 1999
Δ⁹-Desaturase

- Physical Characteristics
- Regulation
  - Endocrine
  - Dietary
  - Physiological State
- Methods of Regulation
  - Transcriptional
  - Microsomal Protease

cis-9, trans-11 CLA Endogenous Synthesis

- mice - Santora et al. 2000
- rats - Ip et al. 1999
- humans - Salminen et al. 1998
  - Adlof et al. 2000
  - Turpeinen et al. 2001
Relative Risk of Coronary Heart Disease in Women

<table>
<thead>
<tr>
<th>Trans Isomer Source</th>
<th>Relative Risk in Quintile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Vegetable fats</td>
<td>1.00</td>
</tr>
<tr>
<td>Animal fats</td>
<td>1.00</td>
</tr>
</tbody>
</table>

¹Nurses health study - 69,181 women

Willet et al., Lancet 341:581
Transfer of Dietary CLA Isomers to Milk Fat in Dairy Cows

<table>
<thead>
<tr>
<th>CLA Isomer</th>
<th>Series 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Series 2&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>trans</em>-8, <em>cis</em>-10</td>
<td>25 ± 6</td>
<td>23 ± 3</td>
</tr>
<tr>
<td><em>cis</em>-9, <em>trans</em>-11</td>
<td>34 ± 8</td>
<td>23 ± 6</td>
</tr>
<tr>
<td><em>trans</em>-10, <em>cis</em>-12</td>
<td>21 ± 5</td>
<td>11 ± 2</td>
</tr>
<tr>
<td><em>cis</em>-11, <em>trans</em>-13</td>
<td>28 ± 4</td>
<td>26 ± 5</td>
</tr>
</tbody>
</table>

<sup>1</sup>Supplement contains CLA isomers as unesterified fatty acids and was abomasally infused to avoid modifications by rumen bacteria.

<sup>a</sup>Chouinard et al., J. Dairy Sci. 82:2737.

<sup>b</sup>Chouinard et al. J. Nut. 129:1579.
Milk Fat Content of CLA

Large variation among individuals, even when physiological and environmental factors are similar
Milk Fat Content Across Dietary Shifts

Peterson et al., 2002
Desaturase Index in Milk Fat Across Dietary Shifts

Peterson et al., 2002
CONCLUSIONS

• *cis*-9, *trans*-11 CLA mainly from endogenous synthesis via $\Delta^9$-desaturase with *trans*-11 C$_{18:1}$ as substrate

• *trans*-7, *cis*-9 almost exclusively from endogenous synthesis whereas other minor CLA isomers are of rumen origin

• enhancing CLA in foods derived from ruminants involves rumen production of *trans*-11 C$_{18:1}$ and tissue activity of $\Delta^9$-desaturase
Rumen

1. Add C18 polyunsaturated fatty acids

2. Inhibit trans C18:1 reductase

3. Prevent isomerase shift

4. Enhance Δ⁹-desaturase

C18:0

C18:1

c-9, t-11 CLA

C18:2

c-9, c-12 CLA

C18:1

c-9, t-11 CLA

t-10 C18:1

t-11 C18:1

t-10, c-12 CLA

c-9, c-12 C18:2

c-9, t-11 CLA

t-11 C18:1

c-9, t-11 CLA

Tissues

Δ⁹-desaturase

Griinari, 2002