Pilot Study: Conjugated Linoleic Acid Reduces Fasting Blood Glucose and Is Inversely Correlated with Leptin in Subjects with Type 2 Diabetes Mellitus

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β-Cell Dysfunction

Insulin Resistance

Type 2 Diabetes Mellitus (T2 DM)
Type 2 Diabetes Mellitus (T2 DM)

- Insulin Resistance
- β-Cell Dysfunction

Complications:
- Nephropathy
- Retinopathy
- Neuropathy
- ASCVD
Prevalence of Type 2 DM in the U.S.

- Sixth leading cause of death
- 17 million or (6%) have Type 2 DM
- Another 7% Americans Exhibit Impaired Fasting Glucose (IFG)
- 6 million Americans remain undiagnosed
- 50% have complications by time of dx
- Average duration of Type 2 DM = 7 years before dx
- 80-90% will need medication eventually
- 48% will require insulin
Costs of Type 2 DM

- $98 Billion lost per year from loss of productivity
- $44 Billion lost to direct costs of medical care
Management of T2 DM

- Diet
- Exercise
- Medication
- Exogenous Insulin Therapy

↓ Adiposity
↑ Insulin Sensitivity
and/or
↑ Insulin Output
Role of Dietary Fats in T2 DM

- Oleic acid may aid in management of glucose and insulin metabolism
  - Christiansen et al., 1997, others
- Some PUFAs may aid in management of dyslipidemias associated with T2 DM
  - Friedberg et al., 1998

- Am Diabetes Assn (2002): Reduce saturated fats to less than 10% calories
Conjugated Linoleic Acid
CLA Delays the Onset of Type 2 Diabetes in ZDF Rats

- ZDF Males, Diets: CON, 1.5% CLA, or TZD Diet
- 2 Weeks

*Biochem Biophys Res Comm 244: 678-682 (1998)*
& M.A. Belury, unpublished data
& Ryder et al., *(Diabetes 2001)*
CLA Mixture Normalizes Impaired OGTT in ZDF Rats

Biochem Biophys Res Comm (1998)
Effect of Dietary CLA on Glucose

Biochem Biophys Res Comm 1998

![Graph showing the effect of dietary CLA on glucose levels. The x-axis represents different groups: Lean, Fatty CON, Fatty CLA, and Fatty TZD. The y-axis represents glucose levels in mg/dl.](image-url)

- Lean group has a lower glucose level compared to the other groups.
- Fatty CON group has a significantly higher glucose level compared to Lean and Fatty CLA groups.
- Fatty CLA group has a lower glucose level compared to Fatty CON and Fatty TZD groups.
- Fatty TZD group has a similar glucose level to Fatty CLA.

Significance levels: *p < 0.05, **p < 0.01.
CLA Reduces Epididymal Fat Mass

Biochem Biophys Res Comm 1998

Epididymal Fat Mass (mg/g)

Lean (CON)  CON  CLA  TZD

*
CLAReducesPlasmaLeptin

Belury and Vanden Heuvel, 1999
# CLA and TZD: ZDF vs. Lean Littermates

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver Index (mg/g)</th>
<th>Hepatic Lipids (mg/g liver)</th>
<th>Epididymal Mass (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-CON</td>
<td>3.9 ± 0.2</td>
<td>24.7 ± 2.0</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>L-CLA</td>
<td>4.3 ± 0.1 *</td>
<td>21.6 ± 4.4</td>
<td>0.6 ± 0.1 *</td>
</tr>
<tr>
<td>L-TZD</td>
<td>4.1 ± 0.3</td>
<td>20.9 ± 0.8</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>D-CON</td>
<td>4.8 ± 0.2</td>
<td>63.1 ± 19.62</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>D-CLA</td>
<td>4.8 ± 0.3</td>
<td>47.0 ± 4.94 #</td>
<td>1.6 ± 0.1 #</td>
</tr>
<tr>
<td>D- TZD</td>
<td>4.1 ± 0.1 #</td>
<td>34.7 ± 8.2 ##</td>
<td>1.7 ± 0.1</td>
</tr>
</tbody>
</table>

M.A. Belury, unpublished
Elucidate the relationship of CLA to improvements in the management of Type 2 diabetes mellitus
Subjects and Methods

- Subjects: Type 2 diabetes --- *no medication* for glucose control
- Block randomization
- Double-blind; CLA supplements (6.0 g / day) vs. safflower placebo, 8 weeks
- 3-Day Diet and Activity Records & 24-Hr Recalls (0, 2, 4, 6, 8 wk)
- Before (Wk 0) vs. During CLA (Wk 8)
  - Anthropometry
  - Blood glucose, Insulin and Leptin
  - Fatty acid composition
# Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>CLA</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>5/6</td>
<td>6/4</td>
</tr>
<tr>
<td>Age (Yrs)</td>
<td>55 ± 14</td>
<td>62 ± 13</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>146 ± 38</td>
<td>134 ± 34</td>
</tr>
<tr>
<td>Body Weight</td>
<td>206 ± 47</td>
<td>173 ± 42</td>
</tr>
</tbody>
</table>

M.A. Belury, unpublished
Supplement Compliance

- Reported compliance 80% minimum
- t10c12-CLA as Marker of Compliance (* P<0.05)

M.A.Belury, unpublished
Objective 1:
To determine the effects of supplemental CLA on fasting blood glucose (FBG), insulin, body weight and leptin in subjects with Type 2 DM
CLA Lowers Fasting Blood Glucose

M.A. Belury, unpublished
Effect of CLA on Fasting Insulin

M.A. Belury, unpublished
Correlation of Plasma CLA and Weight Reduction (Wt ∆)

Body Weight Change (lbs)

CL A (ng/mg)

r = -0.3739
P<0.100

M.A. Belury, unpublished
Negative Correlation Between Plasma CLA vs. Serum Leptin ($\Delta$)

$\text{CLA (ng/mg)}$

$\text{Leptin (\(\Delta\) (ng/ml))}$

$r = -0.4314$
$P < 0.05$

M.A. Belury, unpublished

$\text{CLA}$

$\text{Safflower}$
Biological Activities of c9t11-CLA vs. t10c12-CLA (Synthetic Mixture of CLA) May Differ
<table>
<thead>
<tr>
<th></th>
<th>c9t11</th>
<th>7,9 (c/t)</th>
<th>8,10 (c/t)</th>
<th>t10c12-CLA, Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef(^a)</td>
<td>74.8</td>
<td>15.8</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Cheese(^b)</td>
<td>82.6</td>
<td>8.3</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>CLA Mix</td>
<td>48.7</td>
<td>----</td>
<td>51.3</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Yurawecz et al., 1998
\(^b\) Sehat et al., 1998
Some Biological Activities of c9t11- CLA

- Accumulates extensively in tissues (Belury et al., 1997, Ip et al., 1999, Banni and Belury, unpub.)
- Alters gene expression & affects tissue development (Moya-Camarena et al., 1999, others)
- Inhibits mammary carcinogenesis (Ip et al., 2002)
Objective 2:
To determine the relationship of the serum level of the dietary isomer of CLA, rumenic acid (c9t11-CLA), with body weight and leptin in subjects with Type 2 DM
c9t11-CLA (Δ) vs. Body Weight (Δ)

\[ R = -0.3230 \]
\[ P < 0.20 \]

M.A. Belury, unpublished
c9t11-CLA (Δ) vs. Leptin (Δ)

\[ r = -0.3961 \]
\[ P < 0.100 \]

M.A. Belury, unpublished
Objective 3:

To determine the extent that CLA may act through a mechanism involving peroxisome proliferator-activated receptor-γ (PPARγ)
Distribution of PPARs

- **PPARα**: liver, heart, kidney, muscle
- **PPARβ**: ubiquitous
- **PPARγ1**: adipose tissue, colon, breast epithelium, macrophages, prostate, muscle
- **PPARγ2**: Adipogenesis
PPAR as a Transcription Factor

- Requires Ligand
- Associates with PPAR Response Element (PPRE); DR-1
- Heterodimerizes with RXR (requires 9-cis RA)
- Some Responsive Genes Include
  - FABPs (ap2), Acyl-CoA Oxidase, CYP4A, LPL, negative – ApoCIII
Binding Affinity of c9t11-CLA for LBD of h-PPAR$\gamma$

![Graph showing the binding affinity of c9t11-CLA for LBD of h-PPAR$\gamma$. The x-axis represents (c9t11)-CLA (nM) ranging from 1 to 100,000,000, and the y-axis represents % of Control ranging from 120 to -20. The graph includes error bars and a trend line indicating an IC$_{50}$ value of 7 µM.]

Belury et al., 2002
Dietary CLA Induces aP2 mRNA in Adipose Tissue in ZDF Rats

Biochem Biophys Res Comm 1998
Is the Improvement of Fasting Blood Glucose in T2 DM Regulated by Activation of PPARγ?
Is the Improvement of FBG in T2 DM Regulated by Activation of PPARγ?

Could improvement of FBG be due to downstream metabolites of CLA?
CLA Metabolites

octadecadienoate (c9,t11 or t10c12)
  $\Delta_6$ desaturase *

octadecatrienoate (c6,c9,t11 or c6t10c12)
  elongase

eicosatrienoate (c8,c11,t13 or c8t12c13)
  $\Delta_5$ desaturase

eicosatetraenoate (c5,c8,c11,t13 or c5c8t12c14)
CD 18:3 Isomers in Subjects with T2 DM

M.A. Belury, unpublished
Linoleic acid, C18:2 n6

γ-Linolenic Acid, C18:3 n6

DGLA, C20:3 n6

Arachidionate (AA), C20:4 n6

CLA, C18:2

C18:3

C20:3

Arachidonate (AA), C20:4 n6

*Δ6 Desaturase

Elongase

Δ5 Desaturase

Activate PPARγ?
Inhibiting Δ6 Desaturase Reduces Activation of PPARγ

Belury et al., 2002
Summary: CLA and Diabetes

CLA Supplementation in Subjects with T2 DM:

- Significantly reduced (p< 0.05) FBG
- Fasting insulin & insulin sensitivity ??
Summary: CLA and Weight Regulation

• Correlation coefficients
  – CLA vs. Body weight (r= - 0.3739; P<0.10)
  – CLA vs. Leptin (r= - 0.4349; P<0.05)
• c9t11-CLA weaker inverse correlations with body weight and leptin than total CLA
Improved FBG correlates with Reduced Body Weight

M.A. Belury, unpublished

R = 0.4601
P < 0.05

Fasting Blood Glucose $\Delta$ (mg/dl)

Weight $\Delta$ (lb)
Summary: CLA and Weight Regulation

• Inversely correlations
  – CLA vs. Body weight (P<0.10)
  – CLA vs. Leptin (P<0.05)

• c9t11-CLA weaker inverse correlations with body weight and leptin than total CLA
Some Biological Activities of t10c12- CLA

– Readily forms metabolites in humans (Banni and Belury, unpublished data)
– Alters gene expression (SCD, hr-lipase, others)
– Reduces adiposity in experimental animals (Park et al., 2000)
Conclusions

• CLA may improve FBG via improved insulin sensitivity, body composition and/or leptin levels
• Effect of CLA on leptin suggests a role for adipose tissue in CLA’s effects on FBG
• Isomeric specific effects of t10c12-CLA and c9t11-CLA in regulating FBG and body weight are likely
Future Directions

- Size (N) and duration
- Adipose tissue composition & distribution using MRI
- Isomer & metabolite activity
- Leptin and other hormones involved in food intake
- Mechanism(s) of action at tissue and molecular levels
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Pharmanutrients & Natural
National Cattleman’s Beef Assn