Effects of conjugated linoleic acids (CLA) on tissue response to homeostatic signals and plasma variables associated with lipid metabolism in lactating dairy cows

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ABSTRACT: We conducted a series of experiments to evaluate the effects of conjugated linoleic acids (CLA) on lipid metabolism and energy homeostasis in lactating dairy cows. In all experiments, multiparous Holstein cows in mid to late lactation were abomasally infused with CLA for 5 d. The initial study established that trans-10, cis-12 CLA markedly reduced milk fat yield whereas cis-9, trans-11 CLA, the predominant CLA isomer in milk fat, had no effect. Across the three investigations, infusions of the pure trans-10, cis-12 CLA isomer (3.5 to 14.0 g/d) resulted in a 25 to 50% decrease in milk fat yield and this was energetically equivalent to 6 to 11% of net energy intake. Effects were specific for milk fat as there were little or no changes in feed intake and the yield of milk or milk protein. In Exp. 1, infusing trans-10, cis-12 CLA had no effect on circulating plasma concentrations of glucose, insulin, or leptin. Basal NEFA concentrations were also unaffected, but lipolytic response to an epinephrine challenge was reduced (33%) when cows received trans-10, cis-12 CLA; this minor change in lipolytic response would be consistent with the slightly more positive net energy balance when cows received trans-10, cis-12 CLA. In Exp. 2, infusing differing amounts of trans-10, cis-12 CLA had only minor effects on basal NEFA concentrations, but again cows receiving trans-10, cis-12 CLA tended to have reduced (24%) lipolytic response to trans-10, cis-12 CLA compared to the control period. In Exp. 3, infusing trans-10, cis-12 CLA had no effect on basal glucose concentrations or glucose response to an insulin challenge. The fractional rate of glucose clearance in response to insulin was also not altered by treatment. In summary, the effects of trans-10, cis-12 CLA in lactating dairy cows appear to be specific for the mammary gland, resulting in reduced milk fat synthesis; adipose tissue response to a homeostatic signal regulating lipolysis (epinephrine), whole-body response to a homeostatic signal regulating glucose homeostasis (insulin), and plasma variables associated with lipid metabolism and energy homeostasis were relatively unaffected by treatment with trans-10, cis-12 CLA.

Key Words: Conjugated Linoleic Acid, Lactation, Lipogenesis, Milk Fat

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Introduction

Dietary supplements of conjugated linoleic acids (CLA) reduce milk fat synthesis in cows, pigs, and women (Bauman et al., 2001). Dietary CLA also reduces the body fat content and rates of fat accretion in several species (Jahreis et al., 2000). Trans-10, cis-12 is a specific CLA isomer that alters lipid metabolism in lactating cows (milk fat yield; Baumgard et al., 2000b) and growing mice (body fat content; Park et al., 1999), but the mechanism(s) is unclear. In growing animals, decreased rates of lipid synthesis and increased rates of lipolysis and mobilization have been observed with CLA, and these are potential mechanisms to explain the decrease in body fat content (Park et al., 1997; Ostrowska et al., 1999a). Changes in circulating concentrations of hormones and metabolites associated with energy homeostasis, as well as altered response to homeostatic signals involved in the regulation of lipid metabolism have also been postulated as the basis for the CLA effects in growing animals (Park et al. 1997; Ostrowska et al., 1999b; DeLany and West, 2000).

Certain diets cause milk fat depression (MFD) in dairy cows, and unique conjugated polyunsaturated
fatty acids produced during rumen biohydrogenation, including trans-10, cis-12 CLA, are thought to be involved (Bauman and Griinari, 2001). However, during diet-induced MFD, there is an increase in body fat that is energetically equivalent to the reduction in milk fat (Davis and Brown, 1970). This seems contradictory with the fact that dietary supplements of trans-10, cis-12 CLA reduce milk fat synthesis in lactating animals and body fat accretion in growing animals. To further examine mechanisms by which trans-10, cis-12 CLA reduce milk fat synthesis, we conducted studies with lactating cows to evaluate adipose tissue response to lipolytic and lipogenic stimuli and to monitor systemic concentrations of circulating metabolites and hormones associated with energy homeostasis.

Materials and Methods

Study protocols and procedures for these experiments were approved by the Cornell University Institutional Animal Care and Use Committee. Studies used lactating Holstein cows fitted with rumen cannulas, and cows were housed in metabolic tie stalls in an environmentally controlled room (23°C) with artificial ventilation and a 24-h light regime. Cows were fed a total mixed ration consisting primarily of chopped alfalfa hay as the forage component and cracked shelled corn as the concentrate. Diets were formulated using the Cornell Net Carbohydrate and Protein System (Fox et al., 1992) to meet or exceed the predicted requirements for energy, protein, minerals, and vitamins (NRC, 1989), and ingredient proportions were similar to a previous study (Baumgard et al., 2000b). Cows were given ad libitum access to feed with equal portions of fresh feed given twice daily at 0600 and 1800. Orts were weighed and recorded on a daily basis. Water was available at all times.

Experiment 1

Our objective was to examine the effect of trans-10, cis-12 CLA on circulating concentrations of metabolites and hormones associated with energy homeostasis and to examine the lipolytic response to an epinephrine challenge. We included infusion of cis-9, trans-11 CLA as a comparison, because this isomer has been reported to have no effect on milk fat synthesis (Baumgard et al., 2000b). Three cows (183 ± 8 DIM; mean ± SD) were randomly assigned in a 3 × 3 Latin square design to one of three treatments. Treatments were abomasal infusion of 1) control, 2) 10 g/d of cis-9, trans-11 CLA, and 3) 10 g/d of trans-10, cis-12 CLA. Composition of the CLA supplements (Natural Lipids, Hovdebygda, Norway) are presented in Table 1. High-resolution nuclear magnetic resonance spectroscopy verified that the two CLA supplements were almost exclusively cis-9, trans-11 or trans-10, cis-12 CLA isomers, respectively (M. Aursand and A. Saebø, Natural Lipids; personal communication). Conjugated linoleic acid supplements were emulsified in skim milk prior to each period using a microfluidizer (model 110T; Microfluidics, Newton, MA) as previously described (Chouinard et al., 1999a) and stored at 4°C until infused. Infusion of an equal volume of skim milk served as the control treatment. Infusion periods lasted 5 d with a 7-d interval between periods. As a convenient experimental method to bypass rumen fermentation, a 0.5-mm (i.d.) polyvinyl chloride tubing was passed through the rumen cannula and sulcus omasi into the abomasum, and treatment emulsions were continuously infused as previously described (Chouinard et al., 1999a). Cows were milked, and milk was quantified and sampled at 0600 and 1800 daily. Milk components were analyzed as previously described (Baumgard et al., 2000b).

Each cow was fitted with an indwelling jugular catheter on d 3 of the infusion period. Basal concentrations of plasma NEFA, glucose, insulin, and leptin were determined on d 4 using a pool of the seven plasma samples taken prior to the epinephrine administration. Blood was sampled via the jugular catheter and samples (10 mL) were collected into sodium heparinized tubes (100 IU/mL). Epinephrine challenges (1.4 µg/kg BW) were administered on d 4 of infusion at 0900 and again at 1400. Epinephrine HCl (1 mg/mL; Anpro Pharmaceutical, Arcadia, CA) was administered via the jugular catheter and immediately chased with 10 mL of sterile saline. Blood samples were collected at −45, −40, −30, −20, −10, −5, 0, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 120, 125, and 130 min relative to epinephrine administration. Blood was centrifuged and plasma harvested and stored at −20°C until analyzed. The epinephrine dose was chosen to result in a maximum lipolytic response (Sechen et al., 1990).

Plasma concentrations of NEFA were determined by enzymatic colorimetric analysis (WAKO Pure Chemical Industries, Osaka, Japan) with modifications as previously described (Sechen et al., 1990). The analysis of NEFA was done within a single assay, and the intraassay CV was 2.4%. Plasma levels of glucose were determined by enzymatic colorimetric analysis using a commercial kit (Sigma Chemical, St. Louis, MO). Glucose was analyzed within a single assay, and the intraassay CV was 3.6%.

Plasma concentrations of insulin were quantified by a double-antibody RIA using pancreatic bovine insulin (lot 615-70N-80; Eli Lilly and Company, Indianapolis, IN) for iodination and standards as previously described (McGuire et al., 1995). Plasma leptin concentrations were determined by a homologous RIA using rabbit-anti-recombinant bovine leptin antisera as previously described (Ehrhardt et al., 2000). Both insulin and leptin were analyzed within a single assay, and the intraassay CV were 8.9 and 4.2%, respectively.

Response to epinephrine challenges (area under the curve) was calculated as a linear trapezoidal summation between successive pairs of NEFA concentrations and time coordinates after correcting for the mean baseline NEFA levels. Baseline NEFA concentrations were
calculated as a mean of the seven plasma samples obtained prior to the epinephrine challenge plus the three plasma samples obtained 120, 125, and 130 min after epinephrine administration. Plasma NEFA concentrations peaked at 10 to 20 min and returned to baseline by 45 to 60 min after the epinephrine challenge. To minimize the contribution of clearance and counterregulatory effects, the area under the curve was calculated over the interval from epinephrine administration (0 min) to 20 min post epinephrine injection. Data were statistically analyzed as a 3 × 3 Latin square design using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with treatment, period, and cow included in the model. Data are reported as least square means ± SEM. Orthogonal contrasts were used to test for linear, quadratic, and cubic effect of dose. There were no statistical differences in NEFA response to an epinephrine challenge among the three CLA doses; therefore, animals receiving CLA were analyzed collectively and tested against controls using the ESTIMATE command of SAS.

Experiment 2

Our objective was to extend the investigations of treatment effects on lipolytic response by examining this over a range of trans-10, cis-12 CLA doses. Four cows (228 ± 54 DIM) fitted with rumen fistulas were randomly assigned in a 3 × 3 Latin square design. Treatments were abomasal infusion of trans-10, cis-12 CLA at 1) 0.0 g/d, 2) 3.5 g/d, 3) 7.0 g/d, and 4) 14.0 g/d. The composition of the CLA supplement is presented in Table 1, and treatment values represent the actual daily dose of trans-10, cis-12 CLA that was infused. The CLA supplement was emulsified in skim milk as described in Exp. 1 and doses were abomasally infused continuously for 5 d with a 7- to 8-d interval between periods.

Cows were fitted with jugular catheters on d 4 of infusion and on d 5 administered an epinephrine challenge (1.4 µg/kg BW) at 0900 and again at 1400. Blood samples were obtained and plasma harvested as described in Exp. 1. Plasma concentrations of NEFA were determined as described earlier with intra- and interassay CV being 6.2 and 7.5%, respectively. The area under the curve was calculated and reported as in Exp. 1.

Data were statistically analyzed as a 3 × 3 Latin square design using the PROC MIXED procedure of SAS (SAS Inst. Inc.) with treatment, period, and cow included in the model and reported as least square means ± SEM. Orthogonal contrasts were used to test for linear, quadratic, and cubic effect of dose. There were no statistical differences in NEFA response to an epinephrine challenge among the three CLA doses; therefore, animals receiving CLA were analyzed collectively and tested against controls using the ESTIMATE command of SAS.

Experiment 3

Our objective was to examine the effect of trans-10, cis-12 CLA treatment on whole-body glucose response to an insulin challenge. Four cows (286 ± 54 DIM) fitted with rumen fistulas were randomly assigned in a balanced 2 × 2 crossover design. Treatments consisted of abomasally infusing trans-10, cis-12 CLA (13.6 g/d) or skim milk (control). The composition of the CLA supplement is presented in Table 1. The CLA supplement was emulsified in skim milk as described in Exp. 1. Treatments were abomasally infused continuously for 5 d with a 14-d interval between periods. Mammary biopsies were obtained on d 5 to examine the effects of treatment on mRNA expression of key enzymes involved in milk fat synthesis, and these results are presented elsewhere (Baumgard et al., 2002).

Cows were fitted with jugular catheters on d 3 of infusion and administered an insulin challenge (1.0 µg/kg BW) on d 4 at 0900 and again at 1400. Bovine insulin (26.6 U/mg; lot no. 615-70N-80, Eli Lilly and Company) was initially dissolved to 2 mg/mL in 0.1 M HCl and then diluted to 0.4 mg/mL in sterile saline. The insulin solution was administered via the jugular catheter immediately followed by 10 mL of sterile saline. The blood sampling routine was as described for epinephrine challenges. Plasma concentrations of glucose were determined as described, with the intra- and interassay CV being 5.8 and 6.7%, respectively. Plasma glucose concentrations reached nadir 15 to 30 min after insulin administration. To minimize the contribution of clearance and counterregulatory effects, the response area of plasma glucose to the insulin challenge was calculated from time 0 to 30 min after insulin administration, after correcting for baseline values. The rate of glucose clearance in response to insulin was determined using

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>cis-9, trans-11</th>
<th>trans-10, cis-12</th>
<th>trans-10, cis-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>0</td>
<td>1.3</td>
<td>1.0</td>
</tr>
<tr>
<td>C18:0</td>
<td>0</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>cis-9 C18:1</td>
<td>11.7</td>
<td>1.1</td>
<td>0.4</td>
</tr>
<tr>
<td>cis-9, trans-11 C18:2</td>
<td>73.9 (90.3)</td>
<td>6.4 (6.7)</td>
<td>2.2 (2.3)</td>
</tr>
<tr>
<td>trans-10, cis-12 C18:2</td>
<td>7.9 (9.7)</td>
<td>89.5 (93.3)</td>
<td>94.5 (97.7)</td>
</tr>
<tr>
<td>Others</td>
<td>6.4</td>
<td>1.3</td>
<td>1.6</td>
</tr>
</tbody>
</table>

*The percentage of CLA isomers is presented in parenthesis.
Table 2. Effect of abomasally infusing cis-9, trans-10 or trans-10, cis-12 conjugated linoleic acids (CLA) on production variables (Exp. 1)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>cis-9, trans-11</th>
<th>trans-10, cis-12</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake, kg/d</td>
<td>26.3</td>
<td>25.5</td>
<td>25.7</td>
<td>0.7</td>
<td>0.70</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>31.8</td>
<td>32.0</td>
<td>30.9</td>
<td>0.6</td>
<td>0.32</td>
</tr>
<tr>
<td>Milk fat %</td>
<td>3.22^a</td>
<td>3.44^a</td>
<td>2.36^a</td>
<td>0.52</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>g/d</td>
<td>1,033^a</td>
<td>1,106^a</td>
<td>741^a</td>
<td>48</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Milk protein %</td>
<td>2.95</td>
<td>2.91</td>
<td>2.95</td>
<td>&lt;0.10</td>
<td>0.52</td>
</tr>
<tr>
<td>g/d</td>
<td>930</td>
<td>929</td>
<td>908</td>
<td>14</td>
<td>0.45</td>
</tr>
</tbody>
</table>

*Cows were abomasally infused with either skim milk (control) or 10 g/d of cis-9, trans-11 CLA or trans-10, cis-12 CLA.

**Values represent means from d 4 and 5 of infusion. Superscript letters (y, z) within a row denote significant differences (P < 0.01).

Results

Experiment 1

Abomasally infusing 10 g/d of trans-10, cis-12 CLA reduced milk fat content and yield by 27 and 28%, respectively, whereas the same amount of cis-9, trans-11 CLA had no effect on milk fat parameters (Table 2). Neither CLA isomer affected feed intake, milk yield, milk protein content, or milk protein yield. Across treatments, net energy intake averaged 40.3 Mcal/d, and the reduction in milk fat that occurred with trans-10, cis-12 CLA was energetically equivalent to 2.9 Mcal/d, or about 7% of net energy intake.

Plasma concentrations of glucose, insulin, and leptin were not altered by either CLA isomer (Table 3). Basal concentrations of plasma NEFA were also unaffected, but the lipolytic response to epinephrine challenge was reduced by 33% when cows were abomasally infused with trans-10, cis-12 CLA as compared to the control period (Figure 1, Table 3). Cows infused with cis-9, trans-11 CLA had a lipolytic response similar to that observed in the control period.

Experiment 2

Abomasally infusing 3.5, 7.0, and 14.0 g/d of trans-10, cis-12 CLA reduced milk fat yield and content approximately 25, 33, and 50%, respectively (P < 0.001; data not shown). No effects of CLA dose were observed for feed intake, milk yield, milk protein content, or milk protein yield. Net energy intake averaged 31.8 Mcal/d, and the decrease in milk fat was energetically equivalent to 1.8, 2.4, and 3.6 Mcal/d, or about 6, 8, and 11% of net energy intake for the 3.5, 7.0, and 14.0 g/d doses of trans-10, cis-12 CLA, respectively. An extensive description of dose-response relationships to production parameters and milk fatty acid composition has been previously published (Baumgard et al., 2001).

Across all CLA doses, basal concentrations of plasma NEFA were nearly identical to circulating levels during the control period (Table 4). However, there were minor effects of trans-10, cis-12 CLA dose on plasma NEFA with basal concentrations averaging 109^b, 82^c, 116^{ab}, and 133^{a} μmol/L (P < 0.05) for the 0, 3.5, 7.0, and 14.0 g/d of trans-10, cis-12 CLA, respectively (superscript letters denote significance at P < 0.05). There were no differences in lipolytic response to the epinephrine challenge among the three doses of trans-10, cis-12 CLA (P > 0.5); therefore, they were compared collectively vs control values. Similar to Exp. 1, abomasally infusing trans-10, cis-12 CLA tended to reduce NEFA response to an epinephrine challenge (24%; Table 4). The temporal pattern of NEFA response to the epinephrine challenge was also similar to that observed in Exp. 1 (data not reported).

Experiment 3

Consistent with Exp. 1 and 2, abomasally infusing trans-10, cis-12 CLA (14 g/d) reduced milk fat content and yield by 42 and 48%, respectively (P < 0.01; data not shown). Dry matter intake and milk yield were not affected by treatment, and on average cows consumed 26.6 Mcal/d net energy. The reduction in milk fat secretion when cows received trans-10, cis-12 CLA was energetically equivalent to 2.8 Mcal/d, or 11% of net energy intake. A detailed description of performance effects are presented elsewhere (Baumgard et al., 2002).

Glucose homeostasis and whole-body glucose response to insulin were evaluated. Abomasally infusing
Table 3. Effect of abomasally infusing cis-9, trans-11 or trans-10, cis-12 conjugated linoleic acids (CLA) on plasma variables associated with energy homeostasis (Exp. 1)

<table>
<thead>
<tr>
<th>Plasma variable</th>
<th>Control</th>
<th>cis-9, trans-11</th>
<th>trans-10, cis-12</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>48.0</td>
<td>46.0</td>
<td>47.7</td>
<td>1.2</td>
<td>0.60</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
<td>2.2</td>
<td>2.7</td>
<td>2.7</td>
<td>0.5</td>
<td>0.43</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>3.3</td>
<td>3.2</td>
<td>3.0</td>
<td>0.5</td>
<td>0.72</td>
</tr>
<tr>
<td>NEFA Basal, µmol/L&lt;sup&gt;b&lt;/sup&gt;</td>
<td>134</td>
<td>140</td>
<td>144</td>
<td>6</td>
<td>0.54</td>
</tr>
<tr>
<td>Response, µmol/L/min&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1,564&lt;sup&gt;y&lt;/sup&gt;</td>
<td>1,291&lt;sup&gt;x&lt;/sup&gt;</td>
<td>1,042&lt;sup&gt;x&lt;/sup&gt;</td>
<td>111</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<sup>a</sup>Cows were abomasally infused with either skim milk (control), 10 g/d of cis-9, trans-11 CLA, or trans-10, cis-12 CLA.
<sup>b</sup>Average nonesterified fatty acid (NEFA) concentrations determined on samples obtained prior to epinephrine challenge and at 120, 125, and 130 min samples after epinephrine administration.
<sup>c</sup>Lipolytic response to epinephrine challenge (1.4 µg/kg BW) on d 4 of treatment period. Values represent area for the change in plasma NEFA over the interval of 0 to 20 min following epinephrine administration. Superscript letters (y, z) within a row denote significant differences (P < 0.05).

trans-10, cis-12 CLA had no effect on basal concentrations of plasma glucose (Table 5). Furthermore, the glucose response area to an insulin challenge was not affected by treatment (Table 5). The fractional rate of decrease in plasma glucose was not different between controls and cows abomasally infused with trans-10, cis-12 CLA (Figure 2, Table 5).

**Discussion**

Conjugated linoleic acids have a number of biological effects that may benefit human health and improve animal production. Altering lipid metabolism is one of these effects and dietary CLA supplements cause a reduction in milk fat secretion in lactating animals and decreased body fat accretion in growing animals (see reviews by Baumgard et al., 2000a; Jahreis et al., 2000; Bauman et al., 2001). Various mechanisms have been proposed to explain the effects on milk fat synthesis and body fat accretion (Whigham et al., 2000; Pariza et al., 2001). In the present study using lactating cows, we evaluated changes in whole-body response to homeostatic signals and circulating concentrations of hormones and metabolites associated with energy homeostasis and lipid metabolism. The initial study (Exp. 1) verified our previous results (Baumgard et al., 2000b).

![Figure 1](image-url)  
**Figure 1.** Plasma concentrations of NEFA after an intravenous epinephrine challenge (1.4 µg/kg BW) in Exp. 1. Treatments consisted of abomasal infusions of skim milk (control; ▲), cis-9, trans-11 CLA (◆), or trans-10, cis-12 CLA (■) for 5 d, and challenges were administered at 0900 and 1400 on d 4 of infusion. Values are means, n = 3; SEM for NEFA ranged from 4.6 to 15.2 µmol/L. See Table 3 for statistical comparison among treatments.
Table 4. Effect of abomasally infusing trans-10, cis-12 conjugated linoleic acid (CLA) on lipolytic response to an epinephrine challenge (Exp. 2)

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Control</th>
<th>trans-10, cis-12</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFA, μmol/Lb</td>
<td>Basal,</td>
<td>109 ± 9</td>
<td>110 ± 5</td>
</tr>
<tr>
<td></td>
<td>Response, μmol/L-minc</td>
<td>2,077 ± 190</td>
<td>1,577 ± 134</td>
</tr>
</tbody>
</table>

* Cows were abomasally infused with either with skim milk (control) or 3.5, 7.0, and 14.0 g/d of trans-10, cis-12 CLA for 5 d. Response for the three doses of CLA were analyzed collectively versus the control. Values represent means ± SEM.
* Average nonesterified fatty acid (NEFA) concentrations determined on samples obtained prior to epinephrine challenge and at 120, 125, and 130 min after epinephrine administration.
* Lipolytic response to epinephrine challenge (1.4 μg/kg BW) on d 5 of treatment period. Values represent area for the change in plasma NEFA over the interval of 0 to 20 min following epinephrine administration.

that trans-10, cis-12 CLA reduced milk fat yield whereas the cis-9, trans-11 CLA isomer had no effect. Across the three investigations, abomasally infusing trans-10, cis-12 CLA reduced milk fat yield by 25 to 50% depending on CLA dose, and this was energetically equivalent to 6 to 11% of the net energy intake.

Based on changes in milk fatty acid composition and mRNA abundance of mammary lipogenic enzymes, a primary mechanism by which trans-10, cis-12 CLA reduces milk fat synthesis is by decreasing de novo fatty acid synthesis (Baumgard et al., 2000b, 2002). Decreased rates of lipogenesis have also been suggested to be the mechanism for the reduction in body fat accretion in growing animals (Ostrowska et al., 1999a). This is supported by data showing that CLA reduces the mRNA abundance of lipogenic enzymes including acetyl-CoA carboxylase and fatty acid synthetase in adipose tissue of growing mice (Tsuboyama-Kasaoka et al., 2000).

Altering the factors associated with glucose homeostasis could be involved in the mechanism whereby CLA reduces milk fatty acid synthesis and body fat accretion. Houseknecht et al. (1998b) demonstrated that dietary CLA decreased both plasma insulin and glucose concentrations in the Zucker Diabetic fa/fa rat, and subsequent work verified these effects and attributed them to the trans-10, cis-12 CLA isomer (Ryder et al., 2001). The Zucker Diabetic fa/fa rat spontaneously develops Type 2 diabetes mellitus, which is characterized by hypoinsulinemia, hyperglycemia, and impaired whole-body glucose tolerance. A dietary supplement of CLA improved the impaired glucose tolerance in response to an insulin challenge via increasing insulin-stimulated glucose transport and utilization by adipose tissue and skeletal muscle (Houseknecht et al., 1998b; Ryder et al., 2001).

Table 5. Effect of abomasally infusing trans-10, cis-12 conjugated linoleic acid (CLA) on glucose parameters (Exp. 3)

<table>
<thead>
<tr>
<th>Glucose variables</th>
<th>Control</th>
<th>trans-10, cis-12</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal concentrations, mg/dLb</td>
<td>77.5</td>
<td>77.1</td>
<td>0.9</td>
<td>0.74</td>
</tr>
<tr>
<td>Insulin challengec</td>
<td>Response area, mg/dL-minc</td>
<td>−299</td>
<td>−297</td>
<td>38</td>
</tr>
<tr>
<td>FRC, min × 10³d</td>
<td>−5.5</td>
<td>−4.7</td>
<td>0.7</td>
<td>0.46</td>
</tr>
</tbody>
</table>

* Cows were abomasally infused with either with skim milk (control) or 14.0 g/d of trans-10, cis-12 CLA for 5 d.
* Average glucose concentrations determined on samples obtained prior to insulin challenge and at 120, 125, and 130 min after insulin administration.
* Glucose response to an insulin challenge (1.0 μg/kg BW) on d 4 of treatment period. Values represent area for the change in plasma glucose concentrations over the interval of 0 to 30 min following insulin administration.
* Fractional rate of clearance in glucose concentrations for samples 0 to 20 min after insulin challenge. Values represent slope of natural logarithms of glucose concentrations (mg/dL).
In the present studies, abomasally infusion of \textit{trans}-10, \textit{cis}-12 or \textit{cis}-9, \textit{trans}-11 CLA did not alter basal plasma concentrations of glucose or insulin. Similarly, dietary CLA had no effect on plasma concentrations of glucose and insulin in growing mice (West et al., 2000), growing pigs (Ostrowska et al., 1999b; Ramsay et al., 2001), and adult humans (Medina et al., 2000). In contrast, in other studies, dietary CLA have been shown to increase blood glucose and insulin concentrations in rodents (DeLany and West, 2000; Tsuboyama-Kasaoka et al., 2000), and these authors have suggested that CLA causes insulin resistance. Reduced rates of glucose utilization for lipid synthesis, as would be the case during insulin resistance, could be a mechanism by which CLA reduces body fat. This has been demonstrated in stroma vascular cells isolated from human adipose tissue and cultured with \textit{trans}-10, \textit{cis}-12 CLA (Brown et al., 2001). However, in the present study, abomasally infused \textit{trans}-10, \textit{cis}-12 CLA had no effect on the plasma glucose response to an insulin challenge, indicating that glucose homeostasis was not altered in lactating cows.

Increased rates of lipolysis and fat mobilization from adipose tissue are thought to contribute to the effects of CLA on body fat content. In the case of growing animals, it has been demonstrated that basal concentrations of NEFA were slightly elevated in rats and pigs fed CLA supplements (Ostrowska et al., 1999b; Azain et al., 2000). This has also been demonstrated in 3T3-L1 cells cultured with CLA (Park et al., 1997, 1999). In the present studies with lactating cows, \textit{trans}-10, \textit{cis}-12 CLA had little or no effect on basal plasma NEFA concentrations in Exp. 1 or Exp. 2. This is consistent with our previous work with dairy cows (Baumgard et al., 2000b). Thus, CLA does not appear to be altering basal rates of fat mobilization in the lactating cow.

Increased adipose tissue response to lipolytic agents could partially explain the effects of CLA on body composition. Growing pigs fed a CLA supplement had an increased NEFA response to a \(\beta\)-adrenergic challenge (Ostrowska et al., 1999b), and this was also observed in vitro incubations of adipocytes isolated from rats fed CLA (Pariza et al., 1997). Our investigations with lactating cows demonstrated the opposite. In Exp. 1, treatment with \textit{trans}-10, \textit{cis}-12 CLA reduced (33%) the NEFA response to an epinephrine challenge as compared to the control and \textit{cis}-9, \textit{trans}-11 CLA treatments. Likewise, in Exp. 2 there was a trend (\(P = 0.08\)) for reduced (24%) NEFA response to an epinephrine challenge when cows received \textit{trans}-10, \textit{cis}-12 CLA, although there were no differences in response among doses of CLA. Thus, there is no indication that increased fat mobilization and enhanced adipose tissue response to lipolytic signals are occurring in lactating dairy cows abomasally infused with \textit{trans}-10, \textit{cis}-12 CLA. Indeed, the modest reduction in NEFA response to epinephrine is consistent with the fact that cows receiving \textit{trans}-10, \textit{cis}-12 CLA are in a more positive energy balance due to the reduction in milk fat secretion and thus are probably experiencing increased rates of adipose tissue lipogenesis.

Leptin is a protein secreted from adipocytes that has been implicated in the regulation of food intake, energy expenditure, and whole-body energy balance (Houseknecht et al., 1998a). Feeding CLA has been shown to decrease plasma leptin concentrations in mice and rats (DeLany et al., 1999; Rahman et al., 2001). Similarly, 3T3-L1 adipocytes cultured with \textit{trans}-10, \textit{cis}-12 CLA had reduced mRNA expression and leptin secretion (Kang and Pariza, 2001). However, in Exp. 1, treatment with \textit{trans}-10, \textit{cis}-12 CLA had no effect on plasma leptin levels as compared to the control or \textit{cis}-9, \textit{trans}-11 CLA treatments.

In the present study, the effects of CLA supplementation on response to homeostatic signals and basal concentrations of hormones and metabolites related to energy homeostasis and lipid metabolism were minimal. This is in contrast to the effects observed in many studies with various species of growing animals as previously cited. The reasons for the discrepancies are not entirely clear but several possibilities merit consideration. First is the obvious fact that the physiological state differs and the published literature provides many examples of similarities and differences in lipid metabolism between lactation and growth. Second, species differences in response to CLA constitute a distinct possibility; for example, based upon changes in body composition growing mice appear to be more sensitive to dietary CLA supplements than growing rats (Jahreis et al., 2000). Third, the amount of CLA necessary to substantially reduce milk fat synthesis (0.02 to 0.10% of diet) is very low (Baumgard et al., 2000a) compared to the amount of CLA required to reduce body fat accretion (0.5 to 2.0% of diet). For example, abomasally infusion of a daily CLA dose at about 45 mg/kg BW reduced milk fat yield by 28% (Chouinard et al., 1999b), but it required feeding a similar CLA supplement at a daily CLA dose of approximately 180 mg/kg BW to decrease the body fat accretion of growing pigs by 31% (Ostrowska et al., 1999a) and about 950 mg/kg BW to achieve a 35% decrease in the body fat content in growing mice (DeLany et al., 1999). Terpstra (2001) has suggested dose comparisons of dietary CLA may be more appropriate on the basis of metabolic rate. On a metabolic body weight basis, we calculate that the above cited daily CLA doses represent approximately 225, 540, and 2450 mg/kg 
BW
0.75
corresponding to lactating cows, growing pigs, and growing mice, respectively. Fourth, investigations differed in duration, with most growth studies lasting several weeks as compared to a few days in the present studies with lactating cows. Often in growth studies, changes in circulating metabolites and hormones have been interpreted as representing the mechanism for the CLA-induced reduction in body fat. To the extent that mechanisms are similar in growth and lactation, results from the present study raise the possibility that the changes may merely be a consequence of long-term changes in body fat content. Finally, the present study and others (Park
et al., 1999; Baumgard et al., 2000b) demonstrate that the effects on fat synthesis and accretion are related to specific isomers and some CLA isomers have no effect. Thus, accurate comparisons between studies are difficult because the CLA isomer composition of the supplement can vary extensively.

Implications

Trans-10, cis-12 is a specific isomer of conjugated linoleic acid (CLA) that reduces milk fat synthesis. The effects appear to be specific for the mammary gland in lactating dairy cows. Doses of trans-10, cis-12 CLA, which cause >25% reduction in milk fat yield, have little or no effect on response to homeostatic signals regulating lipolysis (epinephrine) and glucose uptake (insulin) or plasma variables associated with lipid metabolism and energy homeostasis. Thus, it should be possible to use CLA to reduce milk fat synthesis at various stages of the lactation cycle while maintaining normal regulation of whole-body energy homeostasis and lipid metabolism. The ability of cows receiving trans-10, cis-12 CLA to regulate energy homeostasis and lipid metabolism is consistent with the biohydrogenation theory of milk fat depression and the proposed role for this CLA isomer in diet-induced milk fat depression. Of special interest will be long-term studies to determine the whole-body alterations in energy partitioning.

Literature Cited


